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Proceedings

of the

AIR POLLUTION RESEARCH
PLANNING SEMINAR

Held under Auspices of

Air Pollution Medical Program
Division of Special Health Services

and

Air Pollution Engineering Program
Division of Sanitary Engineering Services

December 18, 19, 20, 1956
Cincinnati, Ohio

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services

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These minutes were edited by
Dr. Harry Heimann, Chief,
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P R O G R A M

December 18

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Engineering Air Pollution Research - Mr. V.G. MacKenzie

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- > Data of Interest Developed at the Ozone Conference of the Armour Research Institute, Chicago - Dr. H. Stokinger
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Screening Toxicity Studies on Organic Peroxides and Hydroperoxides - Dr. E.P. Floyd
Toxicity of the Hydrogen Peroxide - Ozone Mixtures - Dr. J.L. Svirbely
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Pathology of Ozone Toxicity - Dr. O.J. Dobrogorski
Mechanisms of Respiratory Irritants - Dr. L.D. Scheel
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Lung Enzymes Affected by Air Pollutants - Mr. J.T. Mountain

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Respiratory Tract Irritation - Dr. Van Murray Sim
Lung Cancer - Dr. P. Kotin
Eye Irritation - Dr. C. Hine, Dr. W. McEwen
Toxicologic Screening of Network Samples - Dr. H.C. Hodge

December 20

Recapitulation of Engineering Program Data
Recapitulation of Medical Program Data

ATTENDANCE LIST
AIR POLLUTION RESEARCH PLANNING SEMINAR
December 18-20, 1956

MEDICAL PANEL

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The Air Pollution Research Planning Seminar was convened at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, at 12:30 p.m., December 18, 1956.

DR. FISHER: We will begin this afternoon session with a presentation by Dr. Chambers who will discuss the agents that are alleged to be responsible for air pollution effects in Los Angeles.

DR. CHAMBERS: I probably am not especially well-fitted to start a discussion of the phase of the hydrocarbon-solar radiation NO₂ phenomenon as it concerns eye irritation. This is so for two reasons: One is that I do not belong to the medical fraternity; and, secondly, that I do not know anything about the subject from first-hand knowledge. As a consequence, all that I can do is to pose some of the problems that are before us in attempting to control eye irritation in the Los Angeles Basin.

Perhaps some of you will raise the question of why, initially, are we concerned with eye irritation? From the standpoint of a Los Angeleno, I think that can be answered readily. In fact, one of my prime spheres of interest in Los Angeles, as Director of Research of the Air Pollution Control District, is that I must develop a program which will serve to allay the constant demands of the public that something be done about the situation, and at the same time be assured that we are doing those things that are good for the public in terms of long-range objectives. As far as the public is concerned, the one thing that is paramount in its mind in relation to air pollution is eye irritation. Whether the people subjectively respond to eye irritation, or unconsciously respond to it as a manifestation of something else that may be more deepseated, I do not know; but I suspect the latter.

In any event, we feel that in the development of our program it is essential, at least in the interest of political equilibrium, that we do something about eye irritation and eye irritants first; simultaneously, or perhaps at a later date, we must develop a substantial program related to more subtle and probably more dangerous air pollution health effects.

The history of eye irritation investigations in Los Angeles is an excellent example of the research problems in air pollution that involves the dichotomy of professional interest. Here is a situation in which we have a response which is physiological, or neurological if you will. On the other hand, we have the primary interest of those in charge of air pollution control that is aimed at engineering and control of the environment. As a consequence of this dichotomy of professional interest, there has developed a series of speculations about the cause, on the one hand, and possibly rather unguided attempts at control, on the other hand. It is necessary, in dealing with a problem like this, to regard it as a problem for solution and to forget that there are professional competences of various types involved. What we need is a solution to this problem,

and not a determination of whether it is a problem to be tackled by physicians or professional engineers. Some day I hope we will eliminate this unnecessary problem at all levels.

When the Los Angeles Air Pollution District was organized in 1942, there appeared in Los Angeles rather frequent episodes of eye irritation and loss of visibility, accompanied by some obvious plant damage, such as leaf-tip burn on irises, yellowing of certain other plants, and typical "silver-leafig" in others, particularly endive and spinach. When all these things occurred simultaneously, it was naturally assumed as an initial premise that all of them were related to a single causative agent. Possibly this is so, even now. We do not know.

Other assumptions were also made. It was inevitable that experts from the East, who had dealt with smog problems and smoke problems, were called in, and it was equally inevitable that the techniques that had been applied in other situations involving smoke--air pollution in the broad sense - - would be applied in Los Angeles. As a consequence, official pronouncements were made as late as in 1950 that as soon as SO_2 and other oxides of sulphur were removed from the atmosphere there would be no more trouble with smog, or with eye irritation and the other manifestations.

We know now that this was not so. The SO_2 concentrations at all times in the Los Angeles Basin are remarkably low, lower than they are in most other cities in the United States. This may be a consequence of a set of reactions that are peculiar to the Los Angeles Basin and may not actually reflect a lower emission of sulfur oxides into the atmosphere. It may mean that there is a faster rate of conversion of these oxides into more oxidized states so that we are getting sulphates instead of SO_2 or SO_3 . There is some evidence for this in the fact that the air sulphate levels, as well as the nitrate levels, are actually higher than in most other industrial areas. But the SO_2 - SO_3 theory had its vogue and for a long time efforts were made to control the sulfur oxides on the theory that this combination of oxides of sulphur, having proved irritating under local conditions elsewhere, was probably causing our trouble in Los Angeles.

That concept had to be discarded and a closer look had to be taken at all the facets of the eye irritation problem to try to see where the trouble lay. One of the first things that was then done involved a rather unsystematic search for gross pathologic changes that might occur in the optic area. To my knowledge nothing of any consequence turned up in that direction. The two optic manifestations, lacrimation and pain are probably subjective phenomena. In the first instance, that of lacrimation, we are dealing with a mechanism that is probably largely reflexive and, therefore, somewhat centrally controlled. In the case of pain, we are obviously dealing with a phenomenon that is monitored by some central system. So, we are dealing with a subjective rather than an objective response, in all probability. The reason I bring this up is because it

has a bearing on our techniques for measuring the eye irritation response in a quantitative way.

The possibility of finding a way of measuring eye irritation which has to be done in order to get a correlation with any suspected causative agent, has been a difficult one. To date I am certain that no satisfactory procedure for a quantitative measure of the eye irritation response has been developed. There are "panel" procedures that have been used, and are being used, successfully in limited types of studies. For example, it is fully possible to control a "panel" group adequately to determine whether or not there is a change in degree of eye irritation from one five-minute period to another, as the result of the property of a given atmosphere. On the other hand, in an attempt to determine whether or not the atmospheric change from one year to the next results in a quantitative change in eye irritation, we must rely on the memory of subjects as to how intense the pain was a year ago. This is a dubious procedure.

The techniques for panel examination have been various. The Air Pollution Control District is now using one or two human subjects in connection with each of its 16 monitoring stations throughout the area. Each subject makes a record hourly of what he believes to be the degree of eye irritation that he feels in terms of "very heavy", "moderate", or "light" eye irritation, or none at all.

The Air Pollution Foundation has established a series of air pollution monitoring panels, chiefly among industrial workers in plants adjacent to these 16 monitoring stations. They have developed a questionnaire type of report for that purpose. The complete analysis of the first year's operation of that panel is now being made. The results should be available within the next 60 days. The University of California at Los Angeles has had, along with the telephone company in the City, what they call a "double-blind panel" study. This is a carefully controlled pair of panels, one balanced against the other. Each is exposed alternately to the same situation through controllable filters which theoretically take the smog out of the atmosphere. I have not seen their data; but I have been informed that, on a scale of 22 grades of eye-irritating intensity, those panels have given results which are reproducible to within three "points" of that scale. If this is true, it is remarkable in view of the psychological factors that are involved and the difference in physiological states and sensitivities of the individuals who comprise the panels.

You may wonder why we worry so much about these panels. I think it would be desirable to find an indirect indicator of eye irritation which we could measure objectively; but, it must be recognized that to establish the validity of an objective indicator, it

must be possible to correlate it with a direct measure of the subjective symptom of eye irritation. Therefore, no matter what we do, we are right back to some method for measuring the subjective irritation response. Even if we could find a biological or a chemical system which would respond in what we believe to be the same quantitative manner as eye irritation, such a system would still have to be correlated with the subjective eye irritation response before we could use it intelligently.

There is a variety of approaches to the problem that I will discuss a little later, none of which is wholly satisfactory. First, however, I want to lay before you some of the types of substances, other than the oxides of sulphur, which have at various times been considered as possibly responsible for the eye irritation response. Let me say at the outset that at present we do not know the identity of the eye irritants. We know that there are certain chemical and physical systems in which an irritant can be produced by manipulation in the laboratory. We know that those systems do exist in the outside air and we, therefore, have reason to suspect that they may account for some part or all of the naturally occurring irritation, but there is no firmly established basis even for that.

Let me list for you the types of substances that have been considered at various times, none of which has been either completely discarded or finally proved to be the cause, or a cause, of the eye irritation. First, there are the oxides of nitrogen. In all probability the concentration of oxides of nitrogen that are required to produce an eye irritative response are too high to justify a conclusion that they are responsible in the Los Angeles area. Then it became obvious in the early measurements of the atmosphere that we have, as in most places, reasonably high concentrations of organic aldehydes, ketones, and acids, some of which are known, from industrial and other exposure studies, to be irritating. Again, the concentrations of each of these substances was apparently much too low to account for the response. Some of the work carried out by the Bureau of Standards called attention to the apparent fact that relatively high concentrations of chlorinated hydrocarbons were occurring in the atmosphere, particularly the group of the approximate mass of trichloroethylene. The validity of that observation is, I think, in some question since the techniques for this determination have been questioned. Nevertheless, the possibility that chlorinated hydrocarbons may enter into the irritative response has been examined theoretically only, and not experimentally, as far as I know; and, here again we come up against the fact that so far as factual information goes, the concentrations of the substances required to produce eye irritation and lacrimation, are usually much higher than the concentrations that have been found in the atmosphere.

Other substances have been investigated. A substance like acrolein can be expected to occur in the Los Angeles atmosphere; as a matter of fact, traces of it have been identified. The measurement of acrolein has not been a very satisfactory procedure; but, it seems evident that the concentrations of acrolein present do not justify a presumption of its importance.

Then we have the whole group of chlorinated aldehydes, and ketones and similar substances, as exemplified by chloracetone, trichloroacetaldehyde, monochloroacetic acid, alpha-chloropropionic acid and chloracetophenone, which were studied by the Stanford Research Institute and the University of California at Riverside, primarily in connection with plant damage but secondarily to determine their irritating properties. While most of these substances produce a degree of plant damage, and at some concentration can produce eye irritation, the concentration levels required for the effect were all far too high.

More recently, a few observations have led to close attention being applied to some of the formyl compounds, particularly formic acid and formaldehyde. In the first place, it was found by Mader that during smog periods the concentration of formic acid in green leaves of plants is usually doubled or tripled. This led to a close examination of the atmosphere for formic acid, and it was found in fairly high concentrations -- concentrations which approached those known to produce eye irritation on direct exposure. Almost simultaneously this substance was found in the atmosphere of Philadelphia by the Franklin Institute. I will return to this material later.

The work of Haagen-Smit, published in the early part of this decade, proved quite conclusively that a set of photochemical reactions occurring in the atmosphere of Los Angeles could be reproduced in the laboratory. It was found that gasoline vapors, particularly those containing some of the unsaturated hydrocarbons (the olefins), when exposed to sunlight in the presence of reasonable amounts of oxides of nitrogen, went through a reaction which resulted in the formation of ozonides of various types (probably peroxides) and, as one product of the reaction, ozone. Haagen-Smit has used or suggested the use of either the total oxidant value of that system or a measurement of ozone as an indicator of the amount of reaction that was occurring. The general assumption was then made, on the basis of a considerable amount of experimental evidence, that organic compounds of the type of the ozonides and peroxides were associated with the eye irritation response. Indeed, it was shown that this system was capable of producing eye irritation. It would seem that a gasoline vapor containing certain types of compounds, particularly

the unsaturated straightchain hydrocarbons and others as well, when exposed to sunlight or to artificial ultraviolet light in the presence of oxide of nitrogen, is capable of producing the eye-irritating reaction in overall concentrations of the chemicals that are equivalent to those actually found in the atmosphere. This was the key point. The emphasis, therefore, was placed on measurement of ozone and of oxides of nitrogen and, as you all know, there has been a great deal of confusion in the use of the term "oxidant" and ozone. In most instances when we measure the things we call "oxidant" -- and, even when we measure the thing we call ozone -- we should define the method of measurement, because none of the methods used heretofore is entirely specific.

More recently the very productive group at the Franklin Institute at Philadelphia, now removed to the Stanford Research Institute in South Pasadena, using its long path infra-red device, has demonstrated the presence of an intermediate compound in this photochemical reaction which they first designated as Compound X, and which more recently they have succeeded in identifying reasonably well as belonging to a class of compounds which they call peracylnitrites. The implication is clear that the oxide of nitrogen binds itself into this intermediate which seems to be the key and most stable step in the cycle of changes which result in the eventual production of fully oxidized hydrocarbons and ozone.

The peracylnitrites present some very interesting possibilities, but their own association with the eye-irritating response has not been established. It is possible to isolate this group of substances although they are extremely reactive. As a matter of fact, the first sample that was prepared and isolated by the Franklin Institute group exploded spontaneously. But it is being handled safely now, so that there is the prospect of determining experimentally whether or not this particular group of substances is, in fact, closely related to the eye irritation response. You will recognize that this substance does not represent a difference in point of view with the Haagen-Smit theory. It actually is a better definition of what Haagen-Smit had described in general; so that we are now getting to a point where we can understand the nature of this reaction and perhaps identify which of the intermediates is actually responsible for the responses.

I would like to go back for just a moment and point out certain things that happened with respect to the appearance of eye irritation. In the first place, it appears that we have two types of eye irritation in terms of chemical stability. The one that has been generally recognized and discussed and has been the basis for most experimental development so far is the one which obviously is related to the type of reaction that was described by Haagen-Smit and is being defined in more explicit terms now by the Franklin Institute

group. That type of reaction always occurs in the daytime. It is of a very transient nature. Whatever it is, it appears to have low stability in the sense that it tends to disappear very abruptly if a cloud passes over the sun, and its concentration drops off in the afternoon as the angle of incidence of the sun changes.

On the other hand, characteristically in the Fall, and in a very pronounced manner during the last few weeks, we have a type of eye irritation which has followed a very different pattern. It has occurred, when there was complete cloud-cover, when there was a reasonable amount of high-level fog with almost no ground-level radiation. In the second place, it has occurred quite frequently immediately following sunset and it persists for a considerable time. On the basis of their behavior, one has to conclude that we are dealing with two types of irritants, one of which has a high degree of stability and another which follows the solar cycle and can be presumed to be of short life.

The short life aspect is of great interest. We have found this year (since we have developed instrumentation capable of time resolution with respect to measurement of ozone, oxidants, oxide of nitrogen and other things), that the oxidant measurement and the so-called ozone measurement, either by potassium iodide or phenolphthalein in the case of oxidant, and by spectrometry or rubber cracking methods in the case of ozone, do not temporally parallel one another in the manner they should if past theories were entirely correct. As a matter of fact, if we are measuring total oxidant, using potassium iodide or phenolphthalein, and we have half a part per million of ozone as measured by the spectrometric or rubber cracking methods, then the potassium iodide or phenolphthalein measurement should obviously give at least that value of total oxidant. We have had numerous episodes this year in which quite the opposite has happened. We had one notable situation, reproduced to a lesser degree several times, in which the potassium iodide total oxidant figure was less than ten parts per million at a time when the ozone measured by the rubber cracking method was at a level of sixty parts per million. This would be impossible if we were actually dealing with ozone. This and other minor observations, plus some theoretical work by Johnston and Cadle at Palo Alto, have led us to develop some projects for the exploration of the possible role of "free radicals" in the smog phenomenon with respect to eye irritation.

It appears, on the basis of theory at least, that some of the organic free radicals have a far greater half-life than we were led to believe earlier. In general, the inorganic free radicals have a half-life of milliseconds or less, whereas some of the organic free radicals, methods for the preparation of which are now known, will

have a half-life of ten minutes or so. It is also notable that many of the reactions which are now demonstrable in the atmosphere, and that result in the production of organic free radicals, are of such a type that the destruction of one of these free radicals results in the production of two more; so that we have a chain reaction type of phenomenon which is in effect self-multiplying as long as the system is energized. Therefore, it is possible, even with a short-lived radical, to get sufficient concentration of these very reactive substances to produce, theoretically, a biologically irritative response. A free radical would, in theory, almost instantaneously remove a hydrogen atom from any organic molecule, so there is a theoretical basis for "triggering" the response.

In addition to the organic free radicals, we have been exploring one other group of compounds which are rather unusual. These are in the class that the combustion chemists usually refer to as the C_xO_y group of compounds. In particular we have been interested in one of the intermediates that have been identified in the combustion of carbon compounds, namely, carbon suboxide, C_3O_2 , a very peculiar substance. It occurs in the process of conversion of carbon compounds to CO_2 and carbon monoxide. It has been demonstrated spectrometrically in the flame of gasoline and other fuels. It is also possible to isolate it, and our staff has actually prepared samples of carbon suboxide recently. They have found that it is reasonably stable, but it also appears that in concentrations of as low as a tenth of a part per million, and probably even as low as a hundredth of a part per million, eye irritation from it is extraordinarily severe. This is only a suggestion. We are going to follow it a little farther. With respect to the first type of eye irritation, that which is extremely transient, it does not appear that this type of substance could be responsible, for the reason that it is stable and for the reason that the rapid, solar-responding irritant appears to be unstable and very transient. On the other hand, with the other type of irritant which seems to be persistent, there is a possibility that compounds of this sort should be explored.

I have referred a good many times to the fact that the concentrations of irritants which have been found in the atmosphere are, in general, in most cases too low to make possible their consideration as the primary irritant. Actually what we are up against in most cases is what Goetz has described as a kinetic paradox. We have concentrations, let us say, of the order of a tenth of a part per million as a maximum in most cases; and yet from the standpoint of kinetic theory, the number of hits per unit area of optic surface would require more hits ordinarily than could possibly be provided by that concentration if the agent were homogeneously distributed in a gas volume. Therefore, the suggestion has been made, and is substantiated by a great deal of evidence, that the particulate or

condensed form of materials in the atmosphere plays a substantial role in the eye irritation phenomenon. Theoretically, the type of condensation necessary to produce this sort of concentration by adsorption can occur under circumstances that exist in the atmosphere. We know that the appearance of eye irritation is normally associated with an increase of particulate matter, with loss of transparency of the air. Furthermore, there is a considerable amount of evidence to indicate that a substance like formaldehyde, for example, which may be essentially nonirritating or nontoxic at one fixed concentration, becomes extraordinarily toxic when a small amount of an aerosol, such as ordinary engine oil, is sprayed into it.

The whole phenomenon of condensation on the surface of particulate matter that occurs in our atmosphere needs exploration and I have a hunch, supported by a very limited amount of experimental evidence at the present time, that the aerosol will be found to play a greater role in eye irritation than we have considered in the past. If we had a condensation of substances existing in the homogeneously dispersed state at a hundredth of a part per million, and if that condensation could occur on the enormous number of two-tenths to four-tenths micron particles which also occur, then one would have the possibility of a high enough local concentration to trigger a physiological-neurological response without running into the kinetic difficulty. The concentration would be produced and, furthermore, there is the other possibility of the potentiating effect of adsorbed irritants which may, in fact, be the same thing.

Our research needs in the eye irritation field are not altogether clear; but some things are definite. We propose that a search be made for some objectively measurable irritation response, something that can be divorced as far as possible from the psychological impact and the variations produced by physiological differences, exploring the possibility of utilizing blink rate, changes in the vascular bed, or changes in the efficiency of visual perception; anything that can be explored that will give us a more objective and a more controllable type of measurement than the present, "Do you feel bad, or don't you" technique.

We propose to push rapidly and suggest that others explore, at least theoretically, the role of the sub-micron particles in the eye irritation response. We hope that the Franklin Institute and others will make a determination of the relationship of the eye irritant to the photochemical cycle of which the peracylnitrites appear to be representative, and we propose to initiate some exploratory work for an assessment of the irritating properties of shortlived or intermediate-lived free radicals.

The following will appear on our Christmas card. I think it

is pertinent and will serve also as a Christmas greeting to this group from the employees of the District.

The eye-smarting lacrimatory effusion
Perhaps may express synergistic collusion
Of gases and dusts and vapors opaque,
Or products derived through a solar clambake;
Oxidic compounds, peracylnitrites,
Free-ranging radicals out for a fight,
Chlorine-soaked molecules up to C_9 ,
Unstable rings and those more benign,
Acids and ketones and aldehydes which
In appropriate places make highballs taste rich,
Microemitic emissions that make
Obnoxious the broiling of garbage or steak,
Diacetyl and suboxide of C,
Acids of S and of N and of P;
Whatever the cause of the trouble turns out,
We are sure that the chemists know what they're about,
Santa Claus' eyes which formerly ran
Will be dry, and his pack free of charts Ringelmann.
With well-founded hope, if obscured by a tear,
We send you Noel and a Happy New Year.

DR. THOMAS F. MANCUSO: We would like to encourage some questions at this time. I believe we have ample time before the next talk, and in order to get the greatest benefit from this meeting, we would like it to be as informal as possible.

DR. HEIMANN: Is there evidence that the eye irritation in Los Angeles became worse after the sulphur dioxide levels were reduced?

DR. CHAMBERS: As a matter of fact, there is no evidence that the sulphur dioxide levels were reduced. The evidence would seem to indicate that the sulphur dioxide levels were, in the first place, never high. They have never burned coal in that area and, as a consequence, while some of the oil that is burned in the production of power has a reasonable amount of sulphur, the sulphur level is much lower than if they burned other fuels. You must also remember that with the oxidizing atmosphere that is produced, SO_2 doesn't exist very long. We get low levels, abnormally low. We get high sulphate levels.

DR. SCHOETTLIN: A physician at U.C.L.A. has developed what he calls a task group which was a good system of measuring visual perception on a time basis where he can also watch fatigue effects. This might be done very well with your eye irritation studies.

DR. CHAMBERS: I haven't talked with that physician about that.

I think Neal Richardson at U.C.L.A. is exploring some similar techniques.

DR. SCHOETTLIN: He is in the same group.

MR. N. E. MANOS: I was impressed with the reproducibility of this subjective measurement of eye irritation. Could you elaborate on what you meant by "reproducibility"?

DR. CHAMBERS: I have been informed that not only do the two groups produce results which are the same within three points, but also that individuals within the groups perform within three points of the same accuracy. I am quoting a verbal report. If we want more evidence on that, I would suggest we ask Neal Richardson, whose work it is. He is in one of the other panels.

DR. HEIMANN: Where will their report appear?

DR. CHAMBERS: You would have to ask him that. He has told me that he would have the manuscript ready within 60 days.

DR. GERRING: In view of the fact that some of these psychological tests have failed, to what extent have ophthalmologists or neurologists that have to do with sensory diseases been consulted; or, to what extent have they become interested in this problem so it can be attacked from the physiological standpoint with respect to the irritation of the eye?

DR. McEWEN: Our Department of Ophthalmology started a project on eye irritation about a month ago. Dr. Chambers mentioned that there was apparently absence of gross morphological changes. I think that is true. In Los Angeles I do not think any ophthalmologist has found evidence of actual eye damage from the smog. In fact, we have evidence that a little smog may be good for the eye for it increases the growth of this epithelial tissue. I think that eye irritation, which is a subjective phenomenon, and accompanied by tearing which is a reflex, could be measured by such things as the Schirmer test; but, such tests in themselves are quite loose tests. If you have a test of the precision you just mentioned, it is a lot better than anything we have, such as tearing tests, that we can measure ophthalmologically.

DR. CHAMBERS: I think that before we conclude that this double blind panel test is as reproducible as it has been reported to be, the data should be examined by some psychologists and others, because other people have attempted essentially the same type of panel study without anything like such reproducible results.

DR. MENDE: In smog, is the particle-size distribution rate varied or within constant limits? You quoted a figure of two-tenths to four-tenths micron. Is this what you always observe or is it varying within larger limits?

DR. CHAMBERS: There is a considerable range of size. There is a size distribution spectrum; but it does appear, on the basis of recent work that has made it possible for us to resolve particle size distributions down to a tenth of a micron, that in terms of numbers the vast majority of particles in the atmosphere during the smog are of the order of four-tenths of a micron.

DR. MENDE: Is there any correlation between particle size and eye irritation?

DR. CHAMBERS: As I say, we do not know.

DR. STOKINGER: Has any source of the carbon suboxide been identified?

DR. CHAMBERS: In theory it should be produced as an intermediate in the oxidation of carbon compounds in the open flame or in the automobile engine. Whether it is actually produced or appears as a transient, we do not know; and, we have no satisfactory analytical procedure for determining its presence in low concentrations. It would have to be done spectrographically, in all probability. Our work with carbon suboxide has only just begun. Perhaps it is a little premature to even mention it; but its extremely irritating property at such low concentrations suggested that it should be investigated. Have you worked with it, Herb?

DR. STOKINGER: No.

DR. HEIMANN: Dr. McEwen, you said that in Los Angeles they have not found any objective evidence of eye damage. Has anybody looked for it in a routine manner, or is this just a "clinical impression"?

DR. McEWEN: This is a general impression held by ophthalmologists.

DR. KOTIN: Experimentally-produced lacrimation does not cause eye damage. We can expose any mammalian species to a lacrimating dose of the mixture that Dr. Chambers described, prepared either by ozonizing gasoline or beginning with gasoline and oxide of nitrogen in a plexiglass chamber, and can repeat lacrimatory episodes ad infinitum, and even on the fiftieth exposure we find no more morphological evidence of irritation than there was on the first. You must

remember that the threshold for morphological change is so great that you would suspect a lot of physiologic alteration well in advance of any morphologic changes.

DR. GAST: Has there been any study for possible hypertrophy of the tear glands?

DR. KOTIN: It was not sought for specifically; but were it there, I think we would have found it. Dr. McEwen mentioned that maybe a little smog is a good thing, and I can not help but at least philosophically, though not practically, agree with him. This is so because there are no changes that you see in response to exposure to realistic concentrations of artificially prepared smog that would not be compatible with a little adrenal-cortical stimulation; at such times you have an animal whose ability to react is certainly at an exaggerated level, in terms of being able to adapt himself to his environment.

DR. CHAMBERS: I might add that we have found one way to alleviate the symptoms of smog, as far as eye irritation is concerned. I am mentioning this because I forgot to make one point clear. We found on human fumigation experiments with ozone recently, that if we have a severe smog that is causing lacrimation and apparent eye irritation and we walk from the outside air into an atmosphere of pure ozone at two parts per million, we get immediate relief. Ozone is not producing the reaction. That is the point I am making.

DR. McEWEN: Just one more question. We had some report about peracids. Would you care to comment on peracids as eye irritants?

DR. CHAMBERS: I am afraid I am not qualified to make any comment on it beyond pointing out that the peracyl compounds, particularly nitrited ones, are according to the Franklin Institute people, key intermediates in the system that contains the irritant. I doubt if I could make any intelligent comment beyond that. Two papers on this subject have now been published, and they are being followed very shortly by two or three more. I think those papers should be studied very closely because they seem to be "breaking the ice" in a number of directions.

DR. MANCUSO: Dr. Chambers, could you indicate to me what work is going on in regard to the biochemical analysis of lacrimation?

DR. CHAMBERS: You mean the analysis of the tears themselves? I do not know of any specific program on that. Do you, Dr. McEwen?

DR. McEWEN: Yes, we had a project under another sponsor on the electrophoretic pattern of tears. The tears have a fairly high pro-

tein content; it is about one-tenth that of plasma, but still is demonstrable in electrophoretic patterns. It seems that the tearing which one has in his eye all the time, is a combination of lacrimal gland and so-called accessory glands secretion. But in irritative tearing, it is primarily from the lacrimal gland and contains a number of unique items, probably an active secretion from the lacrimal gland.

DR. CHAMBERS: What would be the basis for an expectation that there would be a difference in the lacrimatory secretion if the response is a reflexive one, as we assume it to be?

DR. McEWEN: There is possibly a difference, because you have what is known as psychic weeping and you have irritative weeping and they go, some people feel, by different neural pathways. In other words, if you irritate an eye, you may get a different secretion than if you think about a sad subject and cry. However, we have not been able to show any difference between irritative tears and tears of a child who is unhappy. However, down at U.C.L.A. I believe it is Dr. Brunish who finds that on prolonged tearing from sadness, psychic weeping, there is a diminished albumin content.

DR. CAREY: In this discussion of objective measurements for eye irritation it occurs to me that possibly the electrophoretic changes in the tears might be of significance -- for instance, in its content of sodium chloride. I am wondering if the researchers on the West Coast have done any work on that or have any ideas on the use of measurements of sodium chloride as an index of lacrimation.

DR. McEWEN: We have thought about it but have not done work in that direction. It seemed easier to study the proteins than sodium chloride. English workers have found that the sodium chloride content usually goes down with length of time of tearing, and it is apparently a changing function. This would make it a little difficult to study.

DR. CHAMBERS: Would this not be essentially the same as measuring the liquid volume?

DR. CAREY: There may be a change in effusion of sodium chloride with irritation. I do not know. I am not familiar with this particular aspect. But the amount of sodium chloride by dilution might be an index of the degree of lacrimation.

DR. GAST: Wouldn't that bear a relationship to dilution, Dr. Chambers? With respect to tearing or to glandular activity, usually this is physiologically controlled by the sympathetics and parasympathetics and, in general, as I understand it, in the one case you would expect a higher protein or zymogen content, or whatever the composition is; but, in general, the parasympathetics tend to produce

this type of thing, whereas the sympathetics are more apt to produce the more voluminous and, therefore, lower sodium chloride concentration. That type of thing should be possible for measurement.

DR. McEWEN: That is what we tried to do. That was the idea behind comparing some of this irritative crying with "sad" crying. But we found no difference in the secretion from the eye under those conditions.

DR. GAST: You did it in animals by stimulation, such as you would do on salivary glands?

DR. McEWEN: Animals do not cry. They do not cry by irritation nor by psychic stimulation. A bear is the only species that will do psychic crying; but, the only bear we could get was a Himalayan bear, and he did not cry psychically.

DR. CHAMBERS: I wonder if it is completely out of reason to suggest consideration of the possibility of adapting some neuro-physiological procedure to this study of eye irritation in the sense of single fiber or multiple fiber studies of action potentials excited from affected end organs. The techniques have been extraordinarily useful in the analysis of other sensory perceptions. Is it physiologically or anatomically possible to approach it from that point?

DR. KOTIN: Did not Van Harifield at Cal. Tech. try to do that some years ago? I do not know whether he did it only on an exploratory basis; but you might check that.

MR. HATCH: Getting back to your suggestion of fine particles playing a part here: Is this so real that if you remove those particles from otherwise irritating atmosphere, the irritation disappears?

DR. CHAMBERS: All the devices we now have which remove the particles, also remove the irritants; and we have so far been unable to work backwards.

MR. HATCH: If you filter the air adequately, the irritation disappears?

DR. CHAMBERS: That is correct.

MR. HATCH: This could be removal of the particles or removal of the irritant?

DR. CHAMBERS: Yes. It does not resolve the problem at all.

DR. MANCUSO: I think we can move on to the second part of our program and the addresses by Mr. Dohrman Byers and Dr. Herbert Stokinger concerning data of interest developed at the Ozone Conference of the Armour Research Foundation. We will hear first from Mr. Byers.

MR. BYERS: The Ozone Conference held just about a month ago in Chicago was quite a sizable affair. There were approximately 400 persons registered and there were 65 papers on the program. This gives you an idea of the size of the meeting. It was directed mainly towards the technology of commercial applications of ozone.

As you perhaps know, here in Cincinnati we have one of the largest ozone generators in the world. It is used by Emery Industries for the oxidation of certain fatty acids. So, ozone has come into its own as a chemical in industry. It is also being used to some extent for water treatment and treatment of sewage, seemingly with rather good results. There was considerable interest in the matter of ozone toxicity, because of the hazards which might arise from handling it industrially, and also because of the importance which has been attributed to it in atmospheric pollution studies. A great deal of attention was also paid to the possible meteorological significance of ozone.

There were three concurrent sessions at all times. With two of us there, we covered quite a bit, but not all of it. The analytical aspects, strangely enough, dwelt almost entirely on the analysis as related to toxicity, atmospheric pollution and meteorology. The persons interested in industrial applications seemed to be satisfied with established analytical methods.

Supporting what Dr. Chambers has already said, I went to the meeting hoping for the solution on this matter of the determination of ozone; but, I found that, while some progress has been made and some suggestions offered, we still do not have the ultimate method. Present methods of analysis for low concentrations of ozone or oxidants leave us uncertain of what we are getting. The use of potassium iodide in various forms and the use of ultraviolet absorption measurements are still the prevailing methods for the determination of ozone.

We presented a paper on some of our work which had to do with a comparison of the neutral and alkaline potassium iodide procedures. The amount of iodine liberated by an ozone sample is somewhat dependent upon the pH of the iodide solution. The neutral and the alkaline iodide procedures therefore give different results for identical concentrations, if we accept the usual equation in which each mole of ozone liberates one mole of iodine. We showed in our work, using a neutral one percent potassium iodide solution and the alkaline potassium iodide procedure, that there was a difference of about 35 percent

and that in order to correct from results by the alkaline procedure to those by the neutral procedure, one must multiply the former by a factor of 1.5. This does not necessarily say which one is correct. To bring work based upon an analyses by these two different procedures into the correct perspective you have to make the adjustment. You can do it in the reverse way, if you choose. The alkaline procedure has the advantage of being more stable. You can collect a sample, save it, and analyze it some hours later with reasonable reproducibility and precision. The neutral method, for samples which can be analyzed immediately after sampling, is a bit more reproducible than the alkaline.

One interesting thing brought out by Taube of Chicago University, was that this "bugaboo" of hydrogen peroxide being formed in the solution probably has little basis. He made a study of the formation of the hydrogen peroxide by ozone and water, and found that in the absence of the hydrogen ion there is relatively little hydrogen peroxide formed. However, the presence of hydrogen ion, or certain other acid radicals, seems to favor the formation of a slight amount of hydrogen peroxide and may account for the high results obtained in the acidic reactions with potassium iodide.

Fetner and his coworkers of the Georgia Institute of Technology presented two papers, one of which had to do with the action of ozone on *B. coli*. The other reported the analytical study which accompanied their work. They used four, actually five if you wish, methods of determining ozone. They used an acid iodide method at pH 2, a slightly alkaline method at pH 9. They concluded that at pH 9 they got the correct results and that in neutral solution they got high results. Their conclusion was based on the fact that their iodine results at pH 9 agreed with the determinations which they made using the oxidation of ferrous ion to ferric ion, the oxidation of manganese ion to manganic ion, and the oxidation of bromic ion to free bromine. When these were brought into agreement, they corresponded to determinations with the potassium iodide procedure at pH 9.

Regner presented a new recorder, somewhat different from the Littman recorder which is familiar to most of you. Again the method is based on the neutral potassium iodide reaction. The sample is taken into one port and split into two equal streams. In one of these the ozone is destroyed by a furnace at 350 degrees and this stream is used as a reference blank. The two streams are then passed into individual cells containing potassium iodide solution with enough sodium thiosulfate present to take care of any iodine released. The solution is continuously renewed in each cell from a common reservoir and circulates into another pair of cells. Here, current from a pair of electrodes releases an excess of iodine which is sensed by a pair of depolarized electrodes. By means of the sensing electrodes, the

electrolytic current is controlled to give a fixed, slight excess of iodine. The difference between the electrolytic currents on the two sides of the system is then used to calculate the ozone concentration in the sample. Again we have not eluded the potassium iodide reaction.

Essentially the same idea was adapted by Brewed of England to a meteorological study, and they have an apparatus which he says is cheap enough that they can afford to lose four or five of them per week, sending them up in radiosonde balloons. Using a potassium iodide solution and depolarizing electrode set-up, it sends back a signal which they can measure and express as ozone.

Of particular interest to me was a paper by Renzetti who reported on some determinations made in the Los Angeles atmosphere, using a long-path ultraviolet absorption apparatus which he and his co-workers had developed. This apparatus employs three beams at different wave lengths, and the ozone is calculated from the ratio of the various absorptions. He showed results where he graphed the concentration of ozone in parts per hundred million, running up to somewhere around 35 to 50 parts per hundred million against time of day. Using the neutral potassium iodide method, they obtained concentrations which gave a curve something like that (pointing to blackboard) with a peak in the midday falling off in the afternoon and evening. When they used Haagen-Smit's rubber cracking technique they got a similar curve. Although it did not agree exactly, it followed fairly closely. When they used the phenolphthalein procedure, they again got another curve which did not follow the other two exactly, but conformed in general. However, their curve using the ultraviolet absorption, showed much less variation, having only a slight rise coming across like that (pointing to blackboard). They had originally interpreted this as indicative of a positive interference error in the chemical and rubber cracking methods. They have now concluded, however, that it seems improbable that the three different methods, the oxidation of the iodide ion, the phenolphthalein procedure and the rubber-cracking, would agree so well if it were not ozone, or at least the same material being tested. They give as the reason for the difference (lowering) the possibility that peroxyacetylnitrite, which Dr. Chambers mentioned, may serve to produce this effect on the ultraviolet light.

Several spectrographers at the meeting disputed this, saying that this did not stand to reason, that there would accidentally be a compound in the air which would cancel out his reference beams to just the right amount. It shows, however, that ultraviolet light methods are possibly not any more reliable than the others, because of interferences.

Of the suggestions for new and different procedures, there were primarily three. Rene from the University of Nancy, France, reported on some work they had done on the oxyluminescence method. A number

of inorganic materials and organic materials will give off light or luminescence in the presence of ozone. They experimented with several of these and they found that the material known as luminol, 3-aminophthalhydrazide, was possibly the best, at least of those which they had checked. It gave off a definitely measurable and reproducible luminescence when measured with a photomultiplier. They wet small circles of paper, about the size of confetti, with a solution of the material, dried them, then wet them again just before putting them in a glass tube through which the ozone stream was passing. There would be an immediate rise to a maximum luminescence, followed by a gradual decay. They found that measuring their ozone on the basis of the slope of the decay line gave them quite reproducible results. Their work turned out, on questioning, to have been done at levels of 500 parts per million, and higher. Dr. Rene calculated and estimated on the basis of their work that the method could be used at five parts per million as the lower limit. The method, while it may bear investigation at present, is not too promising.

Delman, of the University of New Mexico, gave a paper in which he proposed a reagent, N-phenyl-2-naphthylamine dichlorobenzene, used as a non-aqueous reagent. I believe that the solvent used was chloroform. This reagent gave a colorimetric estimation of ozone with a sensitivity of about one part per million tentatively; he thought that it possibly could be lowered by further work.

Perhaps one of the most promising, although again not a definite solution, was the recorder which has been developed at Armour Research Foundation and was reported by Ohmer. In this they measured the heat of decomposition of ozone when it was passing over a Hopcalite catalyst. Hopcalite has been known for some time and is used in the old M.S.A. Carbon Monoxide Indicator. It is a metallic oxide catalyst, consisting of a mixture of oxides of manganese, silver, and copper, plus several others, I believe, and it will catalytically decompose ozone. Two thermistors, of which one is coated with Hopcalite, are placed in the ozone stream. The uncoated thermistor measures the temperature produced by the decomposition of the ozone. It is a continuous process and independent of sample size. It is entirely dependent on ozone concentration and not on rate of air flow. An elaborate electronic circuit hooked to a recorder interprets the temperature difference in terms of ozone. They have built an apparatus which they believe is operable over the range of 0.5 to 20 parts per million of ozone. Sulphur dioxide, chlorine, and hydrocarbons are said not to interfere, and nitrogen dioxide interferes only slightly. The peroxide and peroxyacids interfere, but they are of an entirely different order of magnitude from those of the reaction of ozone, in that hydrogen peroxide interferes to the extent of about one-tenth or, in other words, about seven percent of its concentration. Cumene is still lower, to the extent of about one percent of its concentration,

and so on, for the others.

VOICE FROM THE FLOOR: What about carbon monoxide?

MR. BYERS: Carbon monoxide in high concentration, would interfere, undoubtedly. To what extent, I do not know. The apparatus holds promise except for that and a few other objections. However, it has not yet been scaled down to the parts per hundred million, in which the air pollution people are interested.

There was a report by Inn on the extinction coefficient of ozone, in which he revised the results which have been used extensively for ultraviolet measurements of ozone. When it was all summed up, it did not amount to much of a change. He wanted to lower the coefficient from 144 to 135 and it is really not greatly significant to our measurements.

There were also papers presented on the concentrations of ozone in the upper atmosphere in relation to meteorological work. One paper was presented by Dr. Stair, Bureau of Standards, and another gentleman disagreed with Dr. Stair about the significance of the data. Dr. Stair said that in following the ozone concentration of the upper atmosphere over New Mexico, he found that it did not correlate with any of the meteorological findings. Immediately this other gentleman said that in Great Britain they had found the contrary and that possibly Stair either was not measuring ozone or something else was wrong. The problem was finally resolved when they agreed that probably the weather does not change as much during the summer months, where the tests were made, in Arizona, as it does in London.

That, in the main, is the story of the discussion of the analytical aspects of the conference. I cannot go into all of the other data presented, such as ozone formation, and the theoretical aspect of electrical discharge. The very first paper we heard by Professor Briner from the University of Geneva was both interesting and in French. The main point that he had to make was that he came to the conclusion that industrially the production of ozone by the use of ultraviolet light, the photochemical production of ozone, was not very efficient as compared with the electrical discharge method.

There was also a great deal on kinetics. The theory and mathematics of these papers were mostly beyond my depth. They seemed to feel that agreement within 50 to 100 percent was good. By comparison our uncertain analytical methods are not so very bad.

One unfortunate thing about the analytical discussions was that everything seemed to come back to the potassium iodide method. Even the measurements by ultraviolet light are based on determining the

ozone by potassium iodide in order to get their basic measurements.

DR. MANCUSO: Are there any questions?

DR. KOTIN: Did you mention that workers at the Georgia Institute of Technology attempted to use microbiological indicators?

MR. BYERS: No. Fetner and some others at the Institute were working on the destruction of B.coli with ozone; actually, they were working with ozone solutions rather than with the gas stream. They found that ozone would kill B.coli and has an effect very much like chlorine, that of having a "break point".

DR. STOKINGER: There were three papers on the toxic properties of ozone delivered at the O₂ Conference: One was from the Armour Research Foundation, another was by Dr. Clamann of the United States Air Force, and the third paper was from our laboratory.

The Armour Research Foundation paper dealt with quite extensive studies on the acute and subacute toxicity of ozone. They have already published some work on the acute response to ozone. Mittler has extended this work now with subacute experiments to include exposures for considerable periods of time; and, one of the particular features of this work that they reported on at this meeting was the addition of a continuous exposure experiment.

One thing, incidentally, that may bother you was that there was an apparent discrepancy in the LD-50's between Mittler's work and ours. I wanted to point out that this is no discrepancy whatsoever. The reason for this is that they have a different way of expressing their ozone concentrations. They found, for example, that the LD-50 for rats for ozone at three hours, was around 20 parts per million. We will see in a moment on the slide that I will show that we found it to be about five p.p.m. Now, that range looks like a tremendous discrepancy, and that we do not agree. I was very anxious to present to them the idea that we had no disagreement. Actually they had an exposure of three hours. Ours was a four-hour exposure. Therefore, the factor representing the difference in terms of hours, was 1.3.

Now, in regard to the analytical factor, Mr. Byers pointed out that there too we have a difference. They used the neutral method and we used the alkaline, and that adds another factor of one and a half. Then they expressed their data in parts per million by weight, and that is about 1.8 times the parts per million by volume that is commonly used in expressing gaseous concentrations. Now, when you multiply all these factors together, you get a total factor of about 3.6. So, if you take their 20 parts per million divided by the factor of 3.6., you come out with 5.5, close enough to our figure of 5. So

$$5 \times 1.8 \times 1.3 \times 1.5 = 17.6$$

$$\text{or } \frac{20}{1.8 \times 1.3 \times 1.5} = 5.7$$

you see that there is really no discrepancy between Mittler's data and ours.

DR. CHAMBERS: Were these experiments done without any physical work factor?

DR. STOKINGER: The animals in these experiments were quietly sitting in the chamber.

MR. KRACKOW: Herb, what was the period of observation? The known exposure was three hours and four hours. Was the post-exposure observation period one, two, or three days?

DR. STOKINGER: Until no further rats died. In ozone exposure studies, it is a characteristic of the response that the animals die within 24 hours, if they are going to die from the ozone exposure. If they die later, you suspect respiratory infection to be the cause rather than ozone itself.

Now, as I said, Mittler also used some continuous exposures amounting to 240 hours at four parts per million, which would be equivalent to somewhat under three of ours, and this gave an LD-20 in mice. This, you see, would correspond to what would be a ten-day continuous smog, if you could get a smog with around two and a half to three parts per million of ozone. I was a little surprised at this result. It seems to us a little unexpected that such a low concentration could cause such a mortality, because that is getting close to the point at which, as we will see later on, practically no edema occurs in the lungs or no obvious injury, at least according to our experience. It is possible, however, that the continuous exposure may have caused this rather high fatality rate.

He also presented some data on rats that had intermittent exposure for 160 hours on a four-hour per day basis, again at four parts per million. This time he noted no change in the lung weight, or any other indication of injury. Since rats and mice are very similar in response to ozone, these results disturbed me because I would think that if he had looked a little closer, he might have found that there were incipient changes going on in the lungs. Up to the present time, he has made very few pathologic studies. I think he is going to include more critical evaluation of the response by histological and other methods later.

He now is using a method for measuring changes in body weight of the animals and he gets a good correlation in the case of rats, but, as I pointed out to him, he was just lucky. In our work with five animal species, we found that the rat was the only species in which there was a correlation between body weight loss and pulmonary injury.

He has done enough work so that now he can estimate safe and injurious concentrations of ozone, for the rat at least. This he does using a three dimensional graph in which he plots exposure time, concentrations, and response. I think that will appear in the Archives of Industrial Health in about February or March of this year. The factor of time is very important, and I will demonstrate this by showing one or two of our own slides to show you how there are a number of factors that affect this very severe toxicity of ozone.

Now I should like to discuss in detail some of the work that we presented at the Armour Research Foundation conference, and then follow by some information on the response of man to ozone exposure.

(A slide was shown.)

The first slide gives you some idea of the very great toxicity that can be produced by relatively small concentrations of ozone. Here you will see the lethal dose for the mouse is about four parts per million by volume. In the rat, it is slightly higher; but, there is a great deal of difference in the susceptibility of another species, the hamster. Now, the rabbit's susceptibility is about the same as that of the hamster. The guinea pig is around that area also, and the dog seems to be even more resistant.

I would like to say in this connection that these results you see here for LD 50, that is the dose that kills half of the animals exposed at these low concentrations, puts ozone in a class of toxic substances that are more toxic than some of the so-called war gases. For example, chlorine that has been used as a war gas is safe for an eight-hour exposure at five parts per million. Ozone, normally, is not thought of as being a highly potent gas, and this evidence belies that point.

The next slide shows the effect of oxides of nitrogen when superimposed on an exposure of ozone.

(A slide was shown.)

The reason that I emphasize this point is because of the fact that there has been a lot of confusion on the role of NO₂ in ozone toxicity. That comes about for two reasons. One is that we all know that nitrogen dioxide is not a harmless gas. We realize that there is the possible development of appreciable amounts of NO₂ in producing ozone, that is in the oxidation of oxygen by an electrical discharge. If you can oxidize oxygen that way, you could conceivably oxidize nitrogen in the same way; and, indeed that occurs. But in most conditions of the development of ozone, at least in the appliances that are available and the several ozone machines we have been able to test, the amounts of NO₂ that accompany ozone are very small.

The table on the blackboard shows what happens if these amounts of NO_2 are not small. As you see here on this chart, the oxides of nitrogen increase vertically downward as the ozone concentration increases to the right. You can see if you go down two lines, that with very small amounts of oxides of nitrogen, less than two-tenths up to a part per million, the lethality increases, as you would expect, progressively with the increased concentration of ozone. Now, taking four parts per million of ozone as a standard concentration, and adding to it increasing amounts of NO_2 , we see that when there is scarcely any NO_2 in it, it comes out six deaths over ten exposed. When you add a little bit of oxide of nitrogen, that doesn't increase the toxicity. Indeed, it reduces it, and we will show you in a moment why that may occur. If you add a slightly greater amount of NO_2 , four to five parts per million, you further reduce the toxicity. It is not until you add what is equivalent to about 14 parts per million of total irritant gases, 10 parts of oxide of nitrogen and 4 of ozone, that you get an appreciable increase in toxicity.

DR. MUSGRAVE: How large is this series of mice?

DR. STOKINGER: What you see there is a typical result of many experiments we have performed. We have done these many times. These are just examples of what occurs.

You can see that, going further with oxides of nitrogen alone, 17 parts per million, 35 parts per million and so forth, there was no indication of lethality from the gas. Indeed, the Army Chemical Corps has shown the LD 50 to be about 65-75 parts per million for rats.

(A slide was shown.)

We know that if you add ozone to NO_2 , you get N_2O_5 , but there has been some question raised by Diggule and Gage, who claim that N_2O_5 is highly toxic, probably more toxic than ozone, and we wanted to investigate this to see if it were true. Here is an experiment done by Mr. Byers and Mr. Saltzman of our laboratory, in which they made pure N_2O_5 by dehydrating fuming nitric acid with P_2O_5 in the presence of ozone. The excess ozone has been removed and this results in a solid mass of white material which we can freeze, and then release by elevating the temperature, for exposure of animals. As you can see here, even at concentrations as high as 50 parts per million, no animals die in a four-hour exposure. That was from pure N_2O_5 . Then we did a little more difficult experiment, shown in the next slide, in regard to ozone and oxides of nitrogen.

(A slide was shown.)

Here we did a similar type of exposure to N_2O_5 , but we did it in

this way: We mixed the ozone at high concentrations, say about a thousand parts per million and NO_2 around that same amount, stoichiometrically. As you can see, it was only when the ozone was at an appreciable concentration (in the first six rows of figures) that we get appreciable mortality. When the ozone concentration was reduced, but at the same time the NO_2 was increased and the N_2O_5 was increased, no toxicity occurred. I think that is ample evidence that the contribution to the toxicity of ozone by associated oxides of nitrogen is not significant. Now that is important because there are many confusing statements about this in the literature. In fact, in a recent book on aviation toxicology, there is a statement which implies a dominant role of the oxides of nitrogen in the toxicity of ozone. It just does not exist as such. I thought I should resolve that point first because this type of exposure occurs in the Los Angeles or oxidant type of smog. The nitrogen oxides arise from the exhaust of automobiles and the proportion is as great, or greater sometimes, than ozone..

Now, as I said before, we studied a number of factors that might influence the toxicity of ozone. We were looking for ways of reducing the effects and we did a very simple experiment on the effect of intermittent exposures.

(A slide was shown.)

As you can see, when you alternate the exposure to ozone and air, you get far less toxic response than when you give a continuous exposure. About three times as many animals die over a four-hour exposure that is uninterrupted as die when you interrupt the toxic gas exposure with almost an equal amount of fresh air. We did this experiment to simulate the condition of the welder who takes a "cigarette break".

MR. KRACKOW: Is this representative of the statistical analysis of the differences between those two situations?

DR. STOKINGER: Yes, I think you can rely on those figures.

DR. SCHOETTLIN: Does this have anything to do with the time-dose? Do you figure those both on the four-hour basis? The second exposure gets a terrific amount more ozone than the first one because one is continuous and one is alternating.

DR. STOKINGER: The total exposure is four hours in each case.

DR. SCHOETTLIN: If you gave the same amount of ozone, continued the experiment for, say, six hours on the first group to give the same dose, would you have the same mortality?

DR. STOKINGER: The dose is the same in each case. There is a

very good linear relationship between time and concentration. It works with concentrations of under 3 parts per million to over 50 parts per million, so we are pretty sure this is a valid way to do the experiment.

MR. MacKENZIE: Could you clarify one point in the first experiment you are reporting? Does that mean that the first one ran for a total of about seven hours?

DR. STOKINGER: That is right. The animals are exposed for 30 minutes, and then have a 20-minute period of rest. This is repeated until the 30 minute intervals add up to a total of four hours.

The next experiment was a simple demonstration of the significance of age.

(A slide was shown).

We took very young mice here and compared them with very old mice. To our surprise, we found that the old mice were far more resistant than the young mice. Now this is not unknown, as we found out later on. There are a number of studies to show that this occurs with sulfur dioxide, for example, and other respiratory tract irritants. Whether this is due to the thickening of the alveolar walls as the animals get older, or other factors, we do not know. But, this result has been verified with rats and other animal species. One might interpret this to mean that the young children might be far more susceptible to pulmonary irritants than older people.

DR. CHAMBERS: What would be the difference in L.D. 50 for the two groups, the young mice compared with the old?

DR. STOKINGER: If you use 18 animals, for example, you get eleven deaths in the case of young animals, against one death out of eighteen in the older animals. We have done this in rats and in other animals, and it is reproducible, providing you get sufficiently old animals.

DR. KOTIN: How young are the young mice?

DR. STOKINGER: Just getting into maturity.

DR. KOTIN: How old are they in weeks?

DR. STOKINGER: I am not sure that I can answer that.

DR. KOTIN: How close to weaning are they?

DR. STOKINGER: Six weeks or less.

DR. KOTIN: A mouse attains maturity relatively sooner than a human does, so there is really no extrapolation possible from the susceptibility of a young mouse and the young human, unless you know the exact week of life.

DR. STOKINGER: It is a matter of a few weeks as against over a year. They do not get much heavier than 35 grams.

DR. MUSGRAVE: What about determining whether a young mouse would have a faster respiratory rate than an old mouse?

DR. STOKINGER: That may be a factor in the picture.

DR. MUSGRAVE: Infants, for example, have a faster respiratory rate.

DR. STOKINGER: That is certainly a factor in the picture; but, I think that there are other factors that may be more important and that the respiratory rate is a minor factor.

DR. MUSGRAVE: Or the metabolism?

DR. STOKINGER: A significant factor may be that the surface of the vascular bed in the lung of the young animals is closer to the air than it is in the older animal. We know that the alveolar walls thicken in older animals.

(A slide was shown.)

This next slide is an attempt to demonstrate the possibility of reducing some of these effects of ozone by some type of medication. With Vitamin C, as you can see here, there was an appreciable reduction in mortality; but it was not a marked one if you expose animals after you medicate them with doses of about 10 milligrams of the vitamin per mouse. You will hear more about this from Dr. Matzen tomorrow. We tried thiolethylamine as a possible agent to reduce the pulmonary edema and hemorrhage that occurs from ozone exposure, on the basis they had had good success with it in high-pressure oxygen exposures where you get similar type of lung response. This was without any effect. As you see, the animals died in greater numbers when they were given the treatment than without the treatment. So then we thought we would try to enhance the Vitamin C effect, and we got hold of a pill that contained Vitamin C plus other reducing substances like panthothenate, glycolate, cysteine and glycine and a few other reducing substances; and, indeed, we were able to reduce the mortality to practically zero. We haven't explored this much farther because we got interested in other factors. But, the interesting thing here, I would like to have you note, is that animals treated with a lethal dose of nitrogen dioxide and then receiving the reducing mixture did not behave in the same

fashion as those dosed with ozone. This seemed to indicate, that there is a different mechanism between the action of NO₂ and ozone on the lungs. This is, I think, an important point to make because you would see very little distinction histologically. We are going to explore this matter later on.

We have already published data on the effect of exercise during exposure, and we have been able to show that some levels, even as low as one part per million, can prove lethal in six hours, provided the animals are made to exercise in a rotating cage even for only a few minutes of each hour. This, we believe, is an important point. I think that any physician would realize that, because you get adrenal cortical responses, as well as the medullary responses, that they work to the detriment of the host by developing additional pulmonary arterial pressure and increasing the edema. Indeed, this is worked out in a very dramatic way by exposing these animals to one part per million when they die within a day, whereas we have experiments going on in which the animals, which you will hear about later on, have withstood some 400 days of exposure to the same concentrations.

We have also shown that there is a tolerance developed to ozone from concentrations that produce either no injury or very slight injury. This may seem odd, as we will show tomorrow, that we can get irreversible damage to the lung and yet also develop this tolerance. With the tolerance that we developed the animal is capable of withstanding several lethal doses within a short time after the initial "protecting" exposure. This tolerance will last from several weeks to several months, provided that the exposure has been for a sufficient time before the challenging exposure.

The way to reconcile this tolerance to lethal effects of ozone and the production of irreversible changes by ozone in the lung is this: That these two phenomena are different ones and the protection against the edema and hemorrhage has nothing to do with the irritative process that ultimately results in fibrosis about the bronchioles. You will hear more about this tomorrow from our pathologist.

I should like now to report very briefly on the work of Dr. Clamann of the Air Force, presented at the International Ozone Conference. He dealt mainly with human responses to ozone. They were concerned with the ozone hazard in high-altitude flying. They calculated, for example, that if you adequately compressed the air at altitudes of about 30,000 feet and above, this would result in concentrations of ozone of 6 p.p.m. in the cabin. This, of course, is an insufferable concentration and will result in pulmonary edema and hemorrhage in the individuals in most instances. They set about seeing if this were true. They exposed five human volunteers for 30 minutes to ozone concentrations of from three to ten parts per million by

weight. They found that the sensitivity was not related to age (but their group consisted of persons between 19 and 54 years of age, which is possibly not a wide enough span).

During the time that these individuals were exposed, in addition to getting the usual signs of a tickling sensation of the nose and throat and of headaches, they developed sharp chest pain at about two and a half parts per million, in about 30 minutes. These individuals were examined and their vital capacity, after short exposures of one hour or so, was found to be reduced by 50 percent and the total lung capacity was also decreased and the residual volume was increased. Dr. Clamann, who subjected himself to these ozone concentrations, was one of the individuals that had the lowest vital capacity remaining after this exposure. I was very surprised to hear from him that within a day or so after the exposure he felt fine; he totally recovered. This does not agree with some reports that have appeared in the literature of some inert gas-shielded arc-welders in New York State that had been exposed to about nine parts per million (maximum) and who had been hospitalized with congested lungs and in whom the effects lasted over a period of several weeks and even months. I think you will see, as a result of some work we will present tomorrow, why these residual effects could occur.

DR. HEIMANN: In those occupational exposures would there be an exposure to a mixture of oxides of nitrogen and ozone?

DR. STOKINGER: In the instance to which I refer, reported by Geil and Kleinfeld, the air was carefully examined for metal fumes, oxides of nitrogen, and hydrogen chloride, and things of that sort, and it was claimed that the amounts present were not in any proportion that would give any of these effects.

MR. KRACKOW: Have we an idea of the comparative lung function test results of the volunteers compared to the industrial exposures?

DR. STOKINGER: No, I believe that lung function tests were not done in the New York State group.

MR. KRACKOW: The Air Force men were exposed at rest?

DR. STOKINGER: That is right. The interesting part is that the industrial group consisted of welders who had ten to fifteen years of experience and this illness developed in the first few weeks of their use of the new welding process.

DR. HEIMANN: It is hard to accept that at its face value. There must have been particulate matter in the air which would complicate the picture.

DR. FISHER: We have other presentations on similar material tomorrow. We will have some time tomorrow afternoon to pick this up again, Dr. Stokinger, if you do not mind.

DR. KOTIN: I would like to correct the misconception that I may have left with some of you. I do believe that the sensitivity to the irritant gases is related to age. I would not want that to be a matter of record; but, I do think that your treadmill experiments, among other things, indicates that it is not the age alone with its increased metabolic rate, etcetera. That is why I think it is important to know the actual age of the animal, rather than to say simply young animals. To begin with you are apparently not using an inbred species, and with that variable in your work, I think, the other variables than can be controlled, such as the weight or age of the animal may not be so important. You said that you would explain the protection that NO₂ apparently gave to the animals exposed to ozone. I would appreciate your doing that.

DR. STOKINGER: When you have ozone in the presence of NO₂ you have the development of N₂O₅, which, in turn, has lower toxicity than either one as far as we have been able to ascertain; so, you reduce the toxicity by chemical combination.

DR. EMIK: Is there any evidence anywhere that there is any genetic species of animal that is susceptible or not susceptible to any of these things?

DR. STOKINGER: We showed that the hamster is certainly about three times less susceptible than other animals studied.

DR. EMIK: It does not specifically make any difference which animal you use; you do not know?

DR. KOTIN: It does, because if you put three contrasting strains of mice in a chamber, you will find a difference in level of mortality response.

DR. EMIK: That is what I want to know.

DR. STOKINGER: Certainly the length of the trachea has a lot to do with the amount of ozone that gets down into the lung. It may be a quarter or a third of that to which the animal is exposed; it may be even a half, depending on the length of the trachea. This is so because ozone is a very reactive material and it is reactive on the way down toward the lungs.

DR. FISHER: Dr. Lodge is Chief of the Chemistry Section of the Air Pollution Program of the Sanitary Engineering Center. You have now

had as background these discussions of ozone problems which relate variously to industrial exposure, to the interests of Air Force personnel, and to our own community air pollution groups. We now need some discussion of these ozone, or oxidant, findings rather generally throughout this country. Dr. Lodge, would you give us some background information?

DR. J. P. LODGE: Taking the title of my talk in its very broad sense, I shall attempt to set up a basis on which one may place any air pollution situation as a point on a line. I have to credit Lewis Rogers for this classification. His suggestion is that we may take two extreme types of air pollution, and we may call these "reducing" and "oxidizing". They represent, of course, two chemical opposites. They also represent the two extremes of air pollution types as far as pollution incidents are concerned, and about which we hear most.

The reducing smog of which London is the chief example is characterized by a preponderance of sulphur dioxide. Sulphur dioxide is its characteristic constituent. This does not mean that it is necessarily the important one, from the toxic point of view; but, we will say it is the characteristically present one.

The oxidizing type, of which Los Angeles pollution is the most important example, is characterized by the presence of ozone, without, at the moment, going into the nature of any other oxidant which may be present. There unquestionably is ozone present in unnatural amounts.

The reducing smog, characteristically, is worse in the early morning. The oxidizing smog is worse in the afternoon. The reducing smog is characteristically a winter phenomenon. The oxidizing smog characteristically is a fall phenomenon. In regard to the physiological response produced, one may say that the oxidation type is associated with irritation of the upper respiratory tract; the reducing type is associated with deeper lung involvement.

I believe that on the basis of these two extremes we might set up a framework in which we could arbitrarily consider the various air pollution situations throughout the Country. Harold Urey, through some very distinguished work, has put forth a theory that in the beginning of the Earth, we had a reducing atmosphere consisting primarily of hydrogen, and that only over a period of many millions of years did a transformation take place to the present oxidizing atmosphere, predominantly an oxygen atmosphere. This may well, also, be construed to be the story of the transition of air pollution in cities where coal burning is the chief source of gaseous pollutants. The reducing type of smog seems to be the usual result; and, the more coal burned per unit area, other things being equal, the more sulphur dioxide and associated products we get. As things become more advanced technologically -- as we become more "civilized" -- we pass to the oxidizing smog of the Los Angeles type, characteristic of a petroleum economy. Thus, I think, it should be possible after study of a city, to place it at some point along the line joining, as it were, London and Los Angeles.

A preliminary study in this regard was made by Dr. Jacob Cholak of the Kettering Laboratory. His findings were limited by the duration

of their study. He spent only a short period of time in each city that he studied. The result, reported a few years ago in the Archives of Industrial Health, showed a series of measurements of sulphur dioxide and oxidant (or ozone) for a sizable number of cities. He showed that even after all of the usual corrections were made, there was a sort of inverse relationship between sulphur dioxide and the oxidizing material. I think this finding may be attributable to a number of causes. One is the fact that there are chemical interferences between the two determinations which are difficult to calibrate out. Another is the fact that if one is burning oil, one does not burn coal. Third is the fact that there are unquestionably some interactions which take place in the gas phase to destroy the one or the other.

The report showed, for example, that Philadelphia, with very high sulphur dioxide content of the atmosphere, had, on the average, the lowest oxidant content of any of the cities measured, after all due correction was made. I think it is an interesting observation even though the studied periods were short. The mean level for Philadelphia for this period was about one part per hundred million of oxidant. This is a very interesting figure, because air unpolluted by anything whatsoever contains somewhere between two and five parts of ozone per hundred million. This means that Philadelphia was putting out enough sulphur dioxide to destroy the natural ozone to a very large extent, and still had SO₂ left over. I think that we need to know a great deal more about the fluctuations in the natural ozone level and how these may affect the superimposed ozone levels caused by man-made pollution.

We find, then, this tendency to move from the reducing sulphur dioxide pollution toward the oxidizing "ozone" pollution, as we move from solid to liquid and gas fuels. I think it is too early to make any judgment of what the effect of this may be in terms of public health. I do not believe that anyone here would be prepared to say that London smog is more or less healthy than is the Los Angeles smog, both of them being equally thick. The concept that I have described represents an evolution which may have to be reckoned with. It also represents a convenient basis for separating the two types.

DR. HEIMANN: I can not agree with the physiological differences that you describe. I think that the information that we have would show no difference in terms of irritation of exposed mucous membranes; that the two groups, if you like, are similar in that both irritate the exposed mucous membranes. In the case of the Donora episode, where we assumed we had the reducing type of air pollution, we also had some eye irritation. I do not believe that there is enough evidence at the present time to say, with any degree of certainty, that the oxidizing type of air pollution such as you have in Los Angeles, does not cause damage to the lungs. Possibly there is a difference in degree, but I am not even sure of that.

DR. LODGE: You are perfectly right. My physiological differentiation was not on the basis of anything deeper than what one hears about primarily, the overt first complaint; and, I think on that basis possibly you would admit this distinction.

MR. ARTHUR C. STERN: I think there is one thing we might look into in this discussion of SO_2 , and its relation to communities which have a sulphur source in the fuel, and that is the recent change in technology that is just becoming evident, and we do not really know entirely what it means. There is a very large part of the fuel burned in public utility power plants, and in some communities it may represent as much as 50 percent of the total amount burned and a very large percentage of the sulphur getting into the atmosphere presumably is SO_2 . Ten years ago power plant practice limited steam temperatures to around 700 degrees and apparently most of the sulphur dioxide went right through the boilers and out as sulphur dioxide. We now have evidence that in modern public utility power plant practice, with steam temperatures of the order of 1,000 degrees and over, the difference of the several hundred degrees results in a catalytic oxidation in the boilers changing SO_2 to SO_3 . This has resulted in SO_3 production of roughly 20 to 30 times of that which we were getting eight to ten years before. With the eventual change of most of power plants from low temperature and low pressure plants -- around the 600 or 700 degree steam temperature -- to the 1,000 degree or over, we are facing the possibility of SO_3 emissions of 20 times and more of that which we have been accustomed to. Now, you can do your own theorizing from here as to just what that is going to mean to us.

DR. CHAMBERS: I am sympathetic with the idea of attempting to classify, as a philosophical operation and as a basis for exploration of the details; but, I am bound to agree with Dr. Heimann at this stage, that a classification of this sort on the basis of the evidence at hand is a little too "pat". The assumption that there is characteristically ozone in the atmosphere of a place like Los Angeles, I think would be better stated if we were to say that that atmosphere has a demonstrated capability for producing an excess of ozone under certain conditions of solar radiation.

It may very well be that if the same amount of energy, in appropriate wave lengths, were applied to the smog over London, one could also get an excess of ozone. We must not forget that the production of ozone in Los Angeles is related not alone to the concentration of pollutants, but also to the intensity of solar radiation. When we have fog or high cloud cover over Los Angeles, there is no ozone; the oxidant does not rise. In fact, when the air oxidant level is high and a cloud passes over the sun, the level goes down. This is not a characteristic of the pollution itself; it is simply a capability of that pollution to produce something once it is acted upon by a

suitable energy source. On that basis one would have to start considering classification of areas in terms of ground radiation rather than in terms of pollution.

The other side of the problem is that even in Los Angeles the story is not entirely "pat". For example, during this past November if one had to perform sampling for ozone, one would have concluded that that atmosphere did not contain ozone in excess of the usual amounts, and therefore one would classify it as a reducing atmosphere, I presume. Actually, we do not have excess of ozone on very many days during the year. In fact, there were very few this past year. We reached a half a part per million of ozone only 10 times during the entire year at any station of the 16, and never at any time at 3 of the 16 locations simultaneously; so that I do not regard the presence of ozone as an attribute of the kind of pollution that we have in Los Angeles. It is simply a capability in that situation for producing a byproduct.

This gets back to another point that I wanted to mention. We have had a number of situations this year in which it has become quite clear that ozone, far from being an indication of an impending pollution "build-up", may signify that we ought to use the appearance of excessive ozone as an indication that the trouble is about over. What happened -- and we have resolved the sequence of events with instrumentation capable of time resolution -- was this: We find that eye irritation parallels a formation of complexes of hydrocarbons with no appearance of excess oxidizing material or ozone until the peak of eye irritation has been reached. Then we begin to build up an excess of ozone, measured by potassium iodide or rubber cracking, and the peak of excess ozone or oxidant occurs an hour and a half to two hours following the beginning of drop-off in eye irritation.

One interpretation that can be placed on this is that the photochemical process that is resulting in the reaction is producing ozone as an intermediate, which is in turn reacting with other hydrocarbons and other substances until a saturation situation is reached. At that point you begin to accumulate an excess of ozone, a by-product of the reaction; and one interpretation, at least, is that the occasion is about over and, in fact, in practice that has proved to be true. More often than not we call our "smog alerts" after people's eyes have stopped weeping, when we call them on the basis of ozone.

The point I am trying to make is, I think it is highly premature to start classifying situations on a philosophical basis until we have a lot more detailed information about the sequence of events that are occurring.

DR. VAN MURRAY SIM: I am inclined to agree with the last gentleman. When you have a true smog, you have an inversion layer,

whether it is under one kind of roof as in Los Angeles, or under the coal roof of London. This makes a very large "gassing chamber" in which any number of compounds are involved.

In reply to the point raised by Mr. Stern, sulphur trioxide certainly is a much stronger irritant under the specialized conditions of London than is SO_2 , because the problem of humidity comes into consideration. When you raise the humidity in the air, SO_3 becomes very irritating. I think that the problem of ozone, while very interesting, is more an indication that the problem is clearing up rather than increasing.

DR. CHAMBERS: This situation is somewhat analogous to that of stream pollution in which one produces a biological oxidation. In the treatment of water pollution you are producing a chemical oxidation and finally reach a point of completion of that oxidation process. There is then an "overshoot" (with air pollution) with production of more ozone. The ozone must be regarded, I think, as a by-product rather than as a fundamental characteristic of the pollution.

MR. CALVIN BOLZE: In Kansas City we have a Kruger oxidant recorder for experimentation. We monitor the outside air there when we are not using it in experiments. If you were to try to place us as a point on the line you hypothesize, we probably would be at just about the zero point as far as oxidant is concerned. At night we find that we actually have between two to four parts of what we call "negative oxidant". This is because the recorder has enough free iodine to give over 100 percent transmission when reduced. These reducing agents are in the neighborhood of two to four parts per hundred million. But during the daytime we actually cross over and we start getting a positive amount of, again, between two to four parts per hundred million. We have had at least one occasion where we reached a level greater than ten. This was a very unusual case. We had an inversion layer that day and, as a consequence, the concentration was way up.

There is another point I would like to mention here, and that is that one hears about the "average" concentration of the atmosphere, "realistic" concentrations and so on. This particular recorder has about a nine-minute response time. We have two chambers which we alternately sample, and it takes nine minutes from the time you turn the switch before you get your true reading. If you have, say, a wind velocity of one mile per hour, this represents about 800 feet of atmosphere that you are sampling, and it will give you an "average" value. Exactly what the true range of concentrations is, that is, whether you have localized pockets which may be ten times as high and other pockets which are considerably less, is a moot question. This should be considered when one goes to set up experiments using so-called "realistic" concentrations.

DR. CHAMBERS: I would like to comment on that. We have a new Kruger device on test now. It is an entirely spectrophotometric machine based on the ultraviolet. It is a rather simple small cell. It appears that it is going to work well. One thing, however, that has been observed already is that when you have a small sample passing through the cell and are recording with a reasonably close-to-instantaneous response, you get evidence of marked stricture, such as you are talking about with respect to ozone. It makes the instrument almost useless for our monitoring purposes because we get variations within less than a minute, all the way from zero to as much as ten parts per million. In other words, the level is all "over the map". It makes it necessary preceding the instrument to put in, either a retention device which will give an integrated sample, or an electronic device on the other end, so that we can get an integrated reading. I would like to hear some discussion on the remarkable micro-stricture with respect to ozone.

DR. FISHER: Dr. Carey, would you like to comment?

DR. CAREY: When measuring the environment of a town or city, we need to consider the internal, or house, and the external environment. Our measurements in Cincinnati indicate that the internal environment of the house, as measured by sulphur dioxide concentrations, may be one-third to one-half that of the outside. Moreover, there appeared to be very long time-lags, possibly six to twelve hours, in the measurements involved between high concentrations outside and inside. These results are still under analysis; but, that is the picture as it appears to us at the moment.

DR. LODGE: I should like a chance to redefend my thesis which I still think has merit. In choosing sulphur dioxide and ozone as characterizing materials, I was not in the least attempting to imply that these had a direct relationship to the physiologically active materials. I was picking them as being chemically outstanding constituents of these mixtures. I believe that Dr. Chambers actually has, in a sense, admitted my thesis that the Los Angeles atmosphere is an oxidizing atmosphere.

DR. CHAMBERS: In the sense that we have more solar radiation than most of the other places we are talking about.

DR. LODGE: But you also have more NO_2 in an active state. That, plus the solar radiation, is the thing that is setting off the reaction.

DR. CHAMBERS: We do not have enough measurements on NO_2 to be sure of that.

DR. LODGE: I think possibly we can now see some further

characteristics of these two extreme types, one being that the oxidizing type depends on sunlight for its activation, whereas the reducing type is substantially independent of light. And, further, that the reducing type is characterized by high humidities while the oxidizing type is characterized by low humidity. These two factors interact, of course, to produce the opportunity for sunlight. I admit that this is an artificial system, and as Dr. Chambers says, it is a philosophical point of view.

I still think it is worthwhile for the reason that when a Londoner speaks of smog, he has a very definite picture in his mind. When a Los Angeleno speaks of smog, he has something very definite in his mind. The two are not the same at all. I know that I have formed a practice that when somebody asks me, what should one do about smog, my first question must be, "what kind of smog". His reply is, "well, smog". Then we have to sit down and present some philosophical basis, such as the one just sketched, for the purpose of explaining what is the outstanding characteristic of what he wishes to attack. On that basis, I think, some such provisional, over-simplified, and, if taken too seriously, misleading classification can be defended simply as a point of departure for describing the different possible effects of these two pollution situations.

DR. CHAMBERS: I have heard one description of the difference between London and Los Angeles smog. In London when they have smog they have fog and smoke. In Los Angeles, when we have smog, we have neither fog nor smoke. If we had either, we would not have eye irritation.

DR. HEIMANN: Did I gather correctly that you feel that, accepting this classification for the moment, in the countries with western culture we are moving from the reducing type of smog towards the oxidizing type of smog?

DR. LODGE: I think this is a fair assumption. Fritz Went certainly subscribes to that.

DR. CHAMBERS: I talked to Fritz Went about this, and it seems that there is another alternative. It is possible that the removal of coal smoke and the subsequent removal of the obstruction of solar radiation is the real thing we are looking at. As we clean up our cities, we are making it possible for the photochemical reaction to occur. We may be saying essentially the same thing; but, the appearance of eye irritation, and similar manifestations, may be an evidence of a new degree of cleanliness in the cities, with respect to smoke.

DR. LODGE: It is always possible to move into the third direction, and away from both by still further policing.

DR. HEIMANN: Is it desirable? Are we policing to get this situation which might be worse than that one?

DR. CHAMBERS: Dr. Langmuir seriously suggested, on his first visit to Los Angeles, that we call in the Army Chemical Corps each day that smog is predicted and have them put up a smoke screen. That would solve the problem.

DR. MUSGRAVE: I was wondering as to the effects of smog on rubber products in Los Angeles and in London; has there been such a comparison made? There has been a lot of complaint in the Los Angeles area about deterioration of rubber, and I had not heard the same complaint from the London area.

DR. LODGE: I know nothing specific from London about that. Haagen-Smit has compiled a list of relative rubber cracking rates for a number of American cities, and the findings are substantially what you would expect. It is highest in the cities that are farthest along toward oxidant air pollution.

DR. CHAMBERS: There again, however, a better correlation exists between the degree of rubber damage and the number of hours of exposure of the atmosphere to direct sunlight than there is to anything else.

DR. FISHER: Dr. Stokinger, do you have any comment? Have you done any further thinking on the relative effects of the extremely high short-time levels as compared with a 24-hour average level?

DR. STOKINGER: If the "bursts" are of very short duration -- I would put it on a probability basis -- they may be of too short duration. I would suggest that there would not be much chance of injuring an appreciable number of cells; whereas if the peaks were of appreciable duration, that is to say for a few minutes or a few respirations, then the peaks could be of great significance physiologically.

DR. FISHER: MR. Krackow, do you wish to comment?

MR. KRACKOW: I was just wondering if these peaks would, in effect, produce an apnea so you would actually be producing a protective type of respiratory pattern.

DR. STOKINGER: That is right.

MR. KRACKOW: So you would actually be defending against the added insult.

DR. HEIMANN: Apnea would occur only if this material irri-

tates the upper portion of the respiratory tract. If it gets down into the lung before it has its irritating action, you probably will not produce apnea.

MR. KRACKOW: This is the question. I think Dr. Stokinger has some tracings of apneic situations which tend to support the idea of this type of irritation producing, in effect, a lower dose retained in the lung.

DR. FISHER: Dr. Sim, did you not have some information on this point that you presented during the Pulmonary Physiology Conference?

DR. VAN MURRAY SIM: Yes. At least with SO_2 and SO_3 , and particularly with SO_3 , you get very little change in final lung volumes; but, you reduce the lung volume when you increase the humidity in the case of SO_3 . You get a marked increase in airway resistance and of the respiratory minute volume as a result of exposure to the highly irritative materials.

MR. HATCH: Of what concentrations of SO_3 are you speaking?

DR. SIM: Well, we have a range of one part per million to 80 parts per million quite a high concentration for a period of an hour.

DR. LESTER SCHEEL: I might make this session a little more confusing than it is by suggesting that possibly when we talk about oxidizing and reducing air pollution, we are talking about something related to the living process which might lie directly in between the two; and, that either might be equally injuring but the response may be pretty much the same on the part of the living organism. I would like, therefore, to hear from plant physiologists for their comment with regard to the Los Angeles smog and, if possible, from someone who knows whether or not plant damage occurs in England.

DR. CHAMBERS: Well, with respect to plant damage in England, Fritz Went, I believe, has apparently detected some in parts of London and Paris. I am not altogether clear whether the type of damage that he found was characteristic of SO_2 smog as we know it in Los Angeles. Does anyone recall that?

DR. VAN MURRAY SIM: I know from a practical point of view, there was legislation in Queen Elizabeth's time that prohibited coal smoke in certain areas of the City of London because of the plant damage, and apparently there is still a lot in the city.

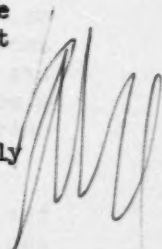
DR. CHAMBERS: Well, it is certainly true that SO_2 produces plant damage, but it is a different type of damage than that produced

by the smog in Los Angeles.

MR. HATCH: As I recall, Went noted that in certain cities he found an increasing amount of plant damage of the type characteristic of Los Angeles over the past several years. He thinks that he can distinguish that type of plant damage from the SO_2 damage.

DR. CHAMBERS: I recall that he found that true in Paris and Rio, and a few other major cities.

DR. KOTIN: A more subtle system, and one which I think is perhaps a little more germane to man and other mammals is using excised segments of the tracheo-bronchial tree, in which you can quantitatively measure the inhibitory or excitatory effect on ciliary movement and actually quantitate mucous membrane activity; you find that the difference between oxidizing substances and reducing substances is a matter of quantity, not quality. So that, at least insofar as the reaction of the respiratory mucosa to particulate deposition is concerned, or just the passage of irritants over respiratory tract cells, there is conceivably little difference. At least on the basis of our own measurements, an SO_2 or an ozone stream over an excised tracheal or bronchial segment, or an SO_2 or ozone atmosphere in a chamber, will interfere in a relatively parallel, if not identical manner with the respiratory tract activity. So in terms of what we are interested in, I think the difference between oxidizing and reducing atmospheres are more of engineering and chemical significance than they are of biologic significance if a particle is going to be retained on a segment of the tracheo-bronchial tree for a prolonged period. Just settling, without instantaneous initiation of removal by ciliary action represents a prolonged period. I think that we can accept the fact that an irritant is an irritant with but little regard for its chemical structure and that the epithelium reacts to an irritant in a relatively non-specific way, in terms of the insult.



DR. CHAMBERS: We are probably dealing with adsorbed substances, action-potentiated perhaps, or concentrated, by condensation on submicron-sized particles. The adsorbed state of certain types of particles, at least in theory, can not be defined as to the state of oxidation that is there. Its state of oxidation is adaptable to the environment in which it happens to find itself; so that to talk about a state of oxidation in the adsorbed state is a little bit nebulous.

MR. HATCH: It seems to me that Dr. Lodge's consideration at this time of a scale of classification that he has suggested is indeed premature. What we are all searching for is, of course, the sharpest description of our problem. But we have talked about Los Angeles versus London, reducing versus the oxidizing agent. There were deaths in London. In contrast eye irritation is the characteristic effect

in Los Angeles. There are remarkable differences in the meteorological situation from one community to another; one with characteristic fog and high humidity versus the other relatively free of fogs. Differences in topography are important. These all add up to wide differences in the rate of ventilation. With respect to what little we know and understand about the London experience, the situation was clearly one in which the state of chronic ill-health of the individuals who were affected was most important. A problem which is serious to chronically impaired people may or may not be serious to people who are not so impaired.

Concerning the mode of action of pollution components, one has to consider the role of carriers, either solid or liquid particulate materials, which we know from practical experience as well as laboratory study, can change a substance from an upper respiratory tract irritant to a deep lung irritant. We know, or we can predict, that the physiologic importance of a unit of irritability in the upper respiratory tract is less than if the same amount of irritation is carried deep into the lungs. So, it seems to me rather dangerous at this time, in attempting to describe the etiology, to skip over all these factors that may make the difference between a serious problem and one which is not serious, much as we would all like to develop a system of classification.

Dr. Kotin suggests that irritation is irritation and perhaps it does not make much difference which end of the scale it is operating on. Irritation may be important to one person and not important to another, in terms of the ultimate effect. So, it seems to me there are many other points here that need to be explored and described in a more succinct way than we have done today before we attempt to categorize problems according to such a scale as has been proposed.

In simple terms, consider the effect of a particular agent: the circumstances under which an individual encounters that agent can, and do, differ to such a degree that at one end of the scale you have no problem and at the other end of the scale you have death. We are dealing here with pollution agents plus a whole host of other factors, and all of these together determine whether we have a problem or not. It is most important that we keep this in mind in our present discussion.

DR. FISHER: We have given consideration to the air pollution after-effects which may result from the Donora episode. To what extent in air pollution can we consider the effect of a single-instance disaster as Donora and its results on the population, as having any effect we can find, measure and study accurately? Can we separate that from the background insult or damage of lesser levels? Dr. Gordon, you were speaking the other day of the results of a single

catastrophe in our air pollution studies.

DR. JOHN E. GORDON: I think the point that I made was that in any disaster --whether it is a fire or flood or a Hiroshima -- to me there is an underlying principle of a division of effect into three phases: First, the course of the immediate disaster; the second, which might be called the delayed effect; and the third, the long-continued after-effect of such a disaster.

To illustrate: In the Hiroshima episode there were the immediate deaths of the disaster. The delayed effects became evident in cataracts and leukemia. And, now, after ten years the interest centers on what is the effect of the lesser grades of exposure to ionizing radiation on the ageing of a population. You could transfer that to a typhoid epidemic, if you will, with the deaths from the immediate typhoid outbreak, second, the intestinal hemorrhages and so forth, and, finally, the gall bladder effect with the production of increased numbers of carriers of typhoid bacillus in the community. It would, therefore, seem to me to be reasonable by such analogies, which one can trace through a long list of disasters, to make use of all the study of what is a possible and to explore the long-delayed effects from such disasters as Donora or the Meuse Valley. I think that expresses my view.

DR. FISHER: Dr. Stokinger, does that stimulate you to any consideration with respect to toxicologic studies?

DR. STOKINGER: Dr. Gordon is speaking of the effects of radiation. Of course, I would place that in a different category from toxicologic response to chemicals. Chemicals have a shorter life span in the body and their effects, of course, are entirely different. The effects of radiation are cumulative and not forgotten, whereas the effects from chemical agents, through their metabolism and elimination from the body, are, by comparison, very short. I can see some possible relationships in terms of exposures from polluted atmospheres, but of an entirely different order of magnitude of time.

DR. MUSGRAVE: I think in the case of benzene there can be a delay of eight to twelve years in the development of aplastic anemia.

DR. STOKINGER: That I think is possible; but they have yet to be proven, I think, in most cases. There are some instances of that type of thing, where they have also been followed by reticular cell sarcomas. There is always a question in those cases where there have been delays, whether some other incident may have caused the response.

MR. KRACKOW: I think the discussion has pointed out the

problem that each disaster is a specific problem in itself. I mean that if it is a polio outbreak, you get certain side effects and after-effects that will live on with people. If it is typhoid, it is something else. If it is due to chemicals, there are different chemicals. If it is the aniline dyes or organic phosphates, the man has his exposure, he recovers, and as far as we know he is perfectly well. Some of these effects that may be significant, may be of a psychological nature, as for example, emotional trauma. This is a big question, and, I think, each disaster has to be studied on its own merits as to the various etiological factors that have produced the immediate effect, the side effect, and the long-range effect.

DR. GORDON: I would like to particularly support that viewpoint, that each problem is individual to itself. But there is a succession of events. You mentioned psychiatric disturbances, and I quote the Coconut Grove fire which had, first, the deaths from the immediate fire the night of the disaster; second, the infections and delayed deaths, and finally, Lindeman's study of the psychologic and psychiatric after-effects of the disaster. True enough, with the nature of the disaster, you determine what is the succession of events in the triad that I have mentioned; but in principle it seems to hold with all disasters.

MR. KRACKOW: I agree that the basic principle is the same.

DR. SCHEEL: I think that this may be the wrong time to bring in the question of air pollution related to injury. I agree with Dr. Stokinger that the primary reaction in injury may be short-lived; but I think we should keep in mind that in air pollution we probably have injury as part of what goes on in our daily living. This injury may be composed of two parts, one that is reversible in the acute stage, and the cumulative but irreversible part that takes place as a result of the continued exposure.

Can we separate the daily environment effect from that of the acute incident? I do not believe that we can collect records over a life span so that we may be able to definitely dissociate the acute incident that causes us alarm with what went on before it.

DR. ROSS L. GAULD: I would like to agree with that. We should look into what led up to the disaster as well as what happened afterwards.

MR. HATCH: The situation in London did not start with that week. There was a fraction of the population that was prepared, for whatever reason, to react badly and that is what we called the disaster. So that what we see in London may have been the end of a disaster rather than the beginning. I am suggesting that the effect of the polluted

air during the severe smog period in London was not the beginning of the problem. It was the effect of that pollution upon damaged organ systems that was characteristic of the episode.

DR. CHAMBERS: In other words, it may not be entirely proper to say that the majority of persons affected in London were not already crippled. It might be more proper to say that air pollution had produced some cripples who were subsequently pushed over the brink.

MR. HATCH: Air pollution or possibly something else. I was so struck with one statement in one of the London reports. It was to this effect: That in respect to the people who died and on whom they had collected various kinds of information, in no instance did they find any evidence of a state of affairs that differed in any significant way whatever from what they see regularly year after year. The only difference was, there were more of them. In other words, these were people who were in an advanced state of disrepair, and that one possible interpretation of this was that there was an insult imposed on an impaired system which was enough to tip them over the edge.

DR. SIM: There is an answer in part to that in the animals of the Smithfield Exposition held during the London smog episode. There were some 450 animals that died and all of the animals came from rural areas outside London, and were not previously exposed. The animals that were in clean stalls were the ones that died with acute pulmonary edema, as far as the veterinary reports went. The pigs and the group who did not have clean stalls, and who had the benefit of the ammonia from their excreta, lived. That is the example, even though what you say is entirely true in relation to people, that it was the young and the old who might have had a previous bronchial infection or long-standing bronchiectasis and were at the point ready to be "tipped over". But, here is the example of these animals being transported to London on the day previous to the fog and so many of them dying.

DR. CHAMBERS: On the other hand, one of the veterinarians who examined a great many of those animals told me that he would consider all of them in a state of "primary insult" in the sense that this was a fat stock show and most of the animals that died were extremely obese. That, too, can be considered an insult.

DR. SIM: The incidence of fat people dying in London was not great, however.

MR. HATCH: One veterinarian told me that these animals were highly developed to make good steaks; but, unfortunately they had not experienced a parallel development with respect to their

respiratory capacity. He spoke, for example, of the bulls that could scarcely run across a field.

DR. EMIK: They are developed to a high extent in order to take "blue ribbons".

MR. HATCH: Perhaps they were respiratory cripples.

DR. EMIK: There are many such animals available in our own country. Maybe we should study them as well. The efforts to get them to win "blue ribbons" eventually led to the selection of individuals. Soon we had the dwarfs, and there are some very interesting physiological varieties produced there. Perhaps these heterozygotes had minor differences from normal individuals.

DR. MANCUSO: I think the trend of the conversation indicates that health studies of urban, suburban and rural areas are indicated, and that if we should get some basic data in regard to the health status of the populations of these different categories, it might serve as base reference points. We do not know enough about general differences in morbidity or mortality patterns.

DR. SCHOETTLIN: In California we have a nursing home reporting service in which we have a population of about 3,000 people in the Los Angeles Basin, who are on the "brink". They are all aged 65 or older. We have been following them for air pollution effects to see if it will be serious for them. Thus far the data are negative. But, when the temperature gets to 100°F., and remains there for even a few hours, their death rate goes up markedly. This, it seems to us, shows that it is a sensitive population, but not necessarily to air pollutants.

DR. HEIMANN: I wonder if I may go back to the difference of opinion that was voiced between Dr. Gordon and Dr. Stokinger. I wonder if the differing points of view are not based on their basic patterns of thinking, each in his individual field. In the case of Dr. Gordon who works with people in the field of epidemiology, he often has to wait a long time to find out what happens to them; whereas, Dr. Stokinger can sacrifice his animals at any time. I believe that is really the difference between their two concepts. In other words, in the case of the animal experiments, if you could let them go on after you have done your acute experiment and wait for the lifetime of a rat, you might find some interesting new data. I wonder whether that is not the basic difference between the thinking of the two.

DR. STOKINGER: No, I do not think that is quite the basis. There is a great mass of information of threshold levels of toxicity

that we have had for a good many years. The limit is low for some substances. But, it is also true that there is a large number of industrial chemicals that have a limit of effectiveness in the body that is not very extreme or not serious, and those are the ones that I think we are dealing with, for the most part, with the smog constituents. We are not always dealing with the very dangerous materials. There are detoxifying mechanisms in the body that get rid of large quantities of these things. They do not remain and do not produce continual insult.

There is a bulk of evidence that does not suggest that all substance from one exposure of a given insult will leave a lasting effect. I think that is the evidence for most chemicals. There are exceptions, of course.

DR. WILFRED DAVID: What about animals with impaired livers? What happens to their detoxification mechanism?

DR. STOKINGER: Well, that is a special case.

DR. PRINDLE: I think the problem here is, perhaps, that Dr. Stokinger is thinking solely of the action of the agent per se; whereas, Dr. Gordon is raising the point that it may have a secondary action. In other words, if we may go to the extreme of postulation and say that perhaps any kind of insult through something like the adaptation syndrome of Selye produces a reaction in the human, and that may produce long-term adrenal activity. The chemical in this case, whatever it may be, has disappeared. But we have long-term effects even to the extreme of psychiatric difficulties, that cannot be necessarily directly attributed to the agent.

DR. STOKINGER: The impression you give me is that you are accentuating the unusual events. In general, those things just do not occur. You can be narcotized with ether for a long time and nothing else occurs. But, I grant you that there are situations like that which you describe. I do not know any good examples where a single exposure to a substance gives an acute effect, an effect that is over in a brief period of time, has then resulted in a response of the body throughout the animal's remaining life. It does not seem to be in agreement with the big mass of evidence we have in medicine.

DR. KOTIN: I cannot agree with you, Dr. Stokinger. In doing long-term carcinogenic experiments, you inevitably attempt to use non-carcinogenic controls; nevertheless, you try to get these controls out of the dirty atmosphere. Not only do you find no evidence of accumulation once the body has metabolized a non-carcinogenic atmospheric pollutant, but more significantly you can submit them all their life to a sub-threshold, or metabolic, exposure and you find

very little effect on longevity. Once a body has metabolized a substance of the type that Dr. Stokinger is discussing, I know of no evidence, and I certainly would love to learn of some, that would indicate he is any less able to handle that second insult than he was the first time.

DR. MUSGRAVE: I can give you an example. An animal sensitized to horse serum and 20 years later given a second dose.

DR. KOTIN: In your example the material (horse serum) has not been metabolized in the sense that you metabolize a toxic agent.

DR. MUSGRAVE: Many an allergenic substance is never metabolized in that sense, and years later you may get an exaggerated response. Not everything is metabolized to the extent that it no longer leaves a trace of having been there.

DR. KOTIN: I do not know that ozone produces sensitivity that can be demonstrable in terms of changes in body proteins, and so on.

DR. CHAMBERS: We have talked a lot about ozone today, but is anybody really concerned with it when we talk about air pollution? The term "carcinogenic substance" has been interjected into the discussion. Frankly I think a lot of us are far more concerned about that than about other matters in relation to air pollution.

DR. FISHER: That is a subject for tomorrow's discussion.

MR. KRACKOW: Actually everyone is right in regard to the point that was raised by Dr. Heimann. I think that when one looks at this matter from strictly the single acute exposure point of view, it will depend on how you design your experiment. Earlier I asked Dr. Stokinger what was his post-exposure observation period. He exposed his animals from three to four hours. It was a one-day observation period. He stopped at that point, because at the end of the second or third day there would be secondary effects which he was not particularly interested in. He designed this experiment from the point of view of the primary effect. On the other hand, we have made some studies in our laboratories where we were concerned about secondary or other effects which might be irreversible. It is a question of how you want to design your experiment and what you are looking for; so actually everybody is right on this.

DR. DONALD M. PACE: I think in this respect you might be interested in some work I am going to report on tomorrow, but I would just as well do it now. We are using different strains of cells in

our tissue cultures. It is interesting to note the different reactions that we get; for example, with sulphur dioxide where the Strain L and HeLa cell which are affected by certain concentrations. They are definitely inhibited, whereas the liver cells are not.

DR. SCHEEL: I would like to come back again to the question of the definition of injury. We have discussed today what you might call total injury, and that is the LD-50 value. In other words, we have killed the animal with the exposure. Then you have injury which you might call cellular injury which results in new tissue taking its place. Now, this pathologic effect is a type of injury which indicates cumulative cellular death. I think that Dr. Pace in his work is interested, not necessarily in cellular death, but in the metabolic changes which may be completely reversible. There are cases, however, where certain metabolic activities can disappear from the cell without involving cellular death. This type of change, again, would have to be classified with the injury that occurs in the irreversible or the cumulative type or the chronic type of injury.

Dr. Stokinger, I think, is interested in toxicity from the point of view of its reversibility. If it is reversible, there is no cumulative damage shown; although of the things that came out in his long-term ozone studies and which surprised him more than anything else, was that he actually did produce pathologic changes as a result of a very low concentration of ozone, repeated many times, and the pathologic changes can only be a cumulative cellular death.

DR. DOBROGORSKI: I do not quite agree that pathology deals only with cell death.

MR. KRACKOW: Although Dr. Stokinger's animals show abnormal tissue changes they have no signs of disease. I mean that functionally they are well. They are healthy to all outward appearances. When the pathologist gets the animals, he finds all sorts of things wrong with them. There are probably some of us here in whom the pathologists would find much disease, even though we are functioning well.

I think that one of the problems we face after a disaster is to determine what percentage of the population is disabled; what proportion of the people is unable to do its job; how many of them have to change from one type of occupation to another. This then is the question of incapacitation versus death in any disaster situation.

DR. MUSGRAVE: Impaired efficiency must also be considered as an effect.

DR. McEWEN: Is this a proper time to ask what in animal studies would be a proper length of time for an acute study and for a chronic study? How long should they be followed?

DR. FISHER: I have been waiting for a chance to ask an opinion on the possibility of setting up colonies, both for acute exposure and long-term exposure periods, some of these animals being physically impaired by surgical procedures. We think that, if we can obtain the funds, we would like to set up such colonies. Would we be justified in trying these in acute experiments, or should we use them in long-term experiments in areas of air pollution? Specifically, we think of using beagles, with perhaps a plastic ring around the bronchus or perhaps tying off a coronary vessel. We can get the operations done for us. What would be the value of an impaired animal colony as a biological sentinel for impending severe air pollution used in the same way that Dr. Schoettlin spoke about the people in homes for the aged in the Los Angeles area?

DR. CHAMBERS: This would be a highly selected group, in the sense that you are arbitrarily impairing all in the same way.

DR. SCHOETTLIN: That is not the only project on the same line. I took a series of bronchial asthmatics aged two and a half to seventy. I studied them through the heavy smog period. Every attack was recorded. We found that there was no correlation with smog.

DR. SIM: If you could produce bronchiectasis or chronic bronchitis in a small group of animals and expose them over a period of time to varying concentrations of air pollutants, keeping all other conditions constant, that could lead to very interesting results. You do not necessarily have to do it in dogs providing, of course, you have a pulmonary disease-free colony to start with.

DR. FISHER: Byerly suggested we either do it with Siamese cats or set up a pure strain of heterozygous alley cats, guaranteed completely heterozygous.

DR. KOTIN: With the help of Dr. Gerring, we did this and used each animal as his own control. We examined the animals for six months with what we call Motley type respiratory profiles and, then we obliterated the pulmonary cavity on one side by the Kulka technique of injecting blood, and obtained oblitative fibrous adhesions between the parietal and visceral pleura. Apparently, in both short-term and long-term exposures, we found that the reserve is so great that neither we nor Dr. Motley could find anything in these animals that indicated that they had been impaired. The measurement made included measuring arterial oxygen, hemoglobin, oxygen saturations,

blood CO₂ and others. Since the dog will not cooperate, you cannot do a ventilation exchange study. In any case, study of blood gases showed no changes at all.

DR. FISHER: Through a generation, Paul?

DR. KOTIN: Oh, no; but, long enough. I would submit that an animal who was his own control for six months, followed by lung space obliteration, and using the same tests again would allow us to make a few assumptions, if not to draw any conclusions.

DR. HEIMANN: The ageing process itself, Paul, is important I think. The process of just getting old, and what that implies in terms of changes in the heart and in the lungs, is very important in terms of considering the effect of even the slight insult of a little irritant air pollution. This is what has come out of the Meuse Valley, Donora and London studies.

DR. KOTIN: That concept would have loaded the experiment in our favor. But, even in spite of it we could find no evidence of really significant changes.

DR. CHAMBERS: On the contrary, the evidence Dr. Stokinger presented would mean that increasing age decreased susceptibility to one type of irritant.

DR. JOSEPH H. GAST: The methodology that Dr. Stokinger has used, the classical approach to toxicological studies, is quite similar to the bacteriological approach. The reason for the selection of the LD-50 is also the reason for the bacteriological basis of dose-response relationship, because there is involved first a challenging agent, secondly, the host. Now, one thing that I think has been lost sight of, in spite of what Dr. Pace mentioned, is the cell itself. The pathologists have already intimated that there are morphological changes. Medicine is finally moving a little away from the specific morphological changes to searching for biochemical changes within the cell. There is a very definite reason for this; and that is, as Dr. Kotin mentioned, the tremendous reserve. You can take any of the organs, the heart, the lungs, the liver, the kidney, and find an enormous physiological reserve. This does not necessarily imply that specific cells have not suffered, have not shown some kind of response to injury, even though the "Easter egg-dye boys" cannot show it. The pathologists are coming around to admit this. There are biochemical and physiological changes. I hope that I can show you some of those tomorrow.

I am not at all impressed by taking the one event, the disaster type of thing. This is somewhat akin to the older studies in bio-

chemistry where you slugged an animal with some material in order to study the chemical changes that took place. I happened to train under that philosophy, so I am very familiar with it. Three to five hundred milligrams of material were fed to an animal, or injected, in order to study the metabolism of that material. Nowadays, we are talking about micrograms of material and techniques have been developed to observe these changes. Therefore, I think, that basically the response of the cell to injury is the particular subject that should always be kept in mind in spite of the fact that we know that the whole organism has a tremendous physiological reserve.

But what happens over a long period of time, which I think was the original point that Dr. Gordon made, when the cells have been subjected to this, even though the toxic agents may be metabolized? I think the history of the sulfonamides is an excellent example of this. Acetylation takes place. There are mechanisms in the body for acetylation but there are also secondary things that occur as a result of it; I do not believe we have even come to the end of that because there are indications of the collagen disease effects of sulfonamides; even though some people have expressed doubt that there are changes in the proteins due to other things than horse serums.

DR. PRINDLE: This is just a question for information. Has anyone attempted a study of this group of elderly people who are allegedly more susceptible, and attempted to find those who are recent additions to the area compared to those who are long-term residents, to see if there is any difference in their reactions?

DR. CHAMBERS: Dr. Breslow's records, I am sure, will include sufficient history of each individual involved so that can be brought out; but, I am not aware that it has been done so far.

DR. SCHOETTLIN: I think it has been done.

MR. HATCH: There is a study in that direction going on here in Cincinnati; Dr. Phair's report on that will come tomorrow.

DR. FISHER: Well, we are well set up for that one when it comes up. Now, you have seen how far we can go with an instrument that gives too sensitive readings.

DR. CHAMBERS: Too sensitive for one purpose. For another purpose it is perfect.

DR. FISHER: I am deeply appreciative of the participation in the discussion. I am personally very happy about the turn it has

taken during the afternoon. There is a background for it. I hope it will emerge a little more clearly tomorrow. Thank you.

(Thereupon, the Seminar was adjourned at 4:30 o'clock, p.m.)

The first speaker this morning was Mr. J. H. Taylor, who presented a paper on "The Role of the Seminar in the Development of the Student's Personality". He discussed the importance of the seminar in the development of the student's personality, and the role of the seminar in the development of the student's personality. He discussed the importance of the seminar in the development of the student's personality, and the role of the seminar in the development of the student's personality.

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Mr. Taylor's paper was a most interesting one, and he presented a very clear and concise picture of the role of the seminar in the development of the student's personality. He discussed the importance of the seminar in the development of the student's personality, and the role of the seminar in the development of the student's personality. He discussed the importance of the seminar in the development of the student's personality, and the role of the seminar in the development of the student's personality.

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The December 19th Session of the Air Pollution Research Planning Seminar was convened at 9:15 a.m., Dr. Wilton M. Fisher presiding.

DR. FISHER: As you will note in the agenda for today, beginning at 10:40 a.m. we start a series of ten-minute presentations. There will be perhaps a few minutes remaining for discussion on those papers. This is a deliberate compromise to give you an idea of what is being done at the Occupational Health Field Headquarters laboratory as a direct operation for our Program.

The first two speakers this morning are men whom we subverted from their proper biological activities into using a combination approach in the study of air pollution. Their contracts were signed and they were able to begin getting to work -- I am using the two words deliberately -- on March 1 of this year. Obviously they have not progressed as far as some of the researchers that have been under way for three, four, and five years. We have been pleasantly surprised by how far they have gone.

Let us consider that the report from these contracting research men is "administrative-confidential." Their results are to be considered only as trends. They are not reporting complete studies. If the man presenting the paper wishes to present that otherwise, he may so specify. I just wanted to call to the attention of this group that from the standpoint of time you are junior workers in the field. It is sort of under duress that I lured you down here to talk today. We have been quite satisfied with the progress you have been making in getting your laboratories under way.

Later in the day Dr. Deichmann from Miami University will be here. Dr. Mende is representing him here this morning. Their laboratory has been in progress for 21 days. We just now got their contract down to them. They will have no report, of course.

The first speaker this morning is Dr. Joseph H. Gast from the Baylor University College of Medicine.

DR. GAST: The contract that I have with the Air Pollution Medical Program has for its primary purpose a search for biological indicators for air pollution effects, using in vitro techniques -- studying cellular materials in the test tube after exposure to polluted air. Therefore, our first objective was the preparation of the pollutants.

At a meeting here in Cincinnati in the Spring, it was decided that the hydrocarbon-nitrogen oxide type of pollutant would be used, and we set about the preparation of this simulated smog. The first slide shows the apparatus that was designed for this purpose.

(A slide was shown.)

We used commercial compressed air, divided the air flow into two parts with one going through nitric acid. The input side of the line is here (pointing). The irradiation unit is here (pointing), and our exposure and testing chamber is on this end of the line. The air is metered through this split line, through the nitric acid to provide the nitrogen oxides, and through the humidity bubbler on this side to provide the humidity for the preparation of what we have called "Haagen-smog", since this simulates his apparatus on a continuously flowing basis.

His preparation, of course, was static. Ours is flowing. Unfortunately, we have not yet solved the problem of getting the hydrocarbon into the system automatically because of the small amounts required. We are using a catalytically cracked gasoline fraction containing about 62 percent olefins, a very reactive material. These all converge here (pointing) in a pre-mix flask which is heated. Then the line is split and we have put in a mechanism to meter the split here, in order to control the rate of flow in the two sides. We have a control line here and our irradiation line or test line on the bottom.

This (pointing) is a 12-liter Pyrex flask and this is a 5-liter Pyrex flask. The slide shows one quadrant of the ultraviolet irradiation unit, utilizing the fluorescent light, as Haagen-Smit had done. Behind these fluorescent lights is placed a special aluminum reflector which does an excellent job of reflecting the light so that we seem to get maximum intensity. There are four of these around each of the flasks. The first one contains 32 of the 15-watt bulbs; the second contains 24.

Then we take off our samples for analysis here. We have tested this here, here and here (pointing) as well as on this side of the exposure chamber. The flow normally goes through our exposure chamber here. We are not using this multiple type sampler now. Originally we thought it would be more convenient and more rapid; but, because of the volume involved and since this is a low pressure, low-flow system, the added volume is too great.

(A slide was shown.)

This is our large type of exposure chamber with a volume of 283 ccm. A synchronous motor (here) causes it to revolve. Our inlet here is constricted and the outlet is bent so that we have no change of flow. We place our cells in here and they are exposed in a thin layer as the chamber turns. Sampling can be done at intervals by putting a pipette in here, collecting the sample after stopping the apparatus, without stopping the flow. This is one type, and we have one each on the control and test lines.

The next slide shows another type of exposure chamber that was designed.

(A slide was shown)

It consists of multiple small flasks on a rotating device. The inlet here bends and goes to each of the five flasks without passing through one flask before going to the next. It passes through this flask right to here and out. We have a motor and a device for changing the rate of oscillation, and this is a simple aluminum clamp over the top of this line. All of this is Pyrex, so that there is no place where there is any contact with anything but glass and the silicone greases that are used on the joints.

We have run various air flows in this system, from four to eight hundred cc's per minute, residence times in the irradiation flask ranging from 34 to 70 minutes. One of the advantages (or disadvantages) of the system happens to be that in our irradiation unit we get high temperatures, between 60 and 80 degrees, so we have operated as nearly as we could control them, at 60, 70 and 80 degrees. In the control line we compensate for this temperature increase of the test line by the use of glass-coiled heating mantles, so that the control represents a heated hydrocarbon-nitrogen oxide mixture which serves as an excellent control on the effect of irradiation. Equilibrium time to reach constant analytical values ranges from three to four hours, depending upon the irradiation-flask residence time.

Because of the absence of any reference data for such a study, and in order to obtain some positive results, we started with relatively high concentrations. We run from four to eight parts per million oxidant, calculated as ozone by the phenolphthalein test, and we have mostly discontinued the potassium iodide testing although we have done both neutral alkaline and potassium iodide in some of our analyses. We use phosphoric acid as the humidifier, as Haagen-Smit reported, and this gives us between 15 and 20 percent humidity. If we substitute water for the phosphoric acid we get from 80 to 100 percent humidity, all calculated to 25 degrees. Our room temperatures vary from 23 to 27 degrees, so we have used 25 degrees as a mean. In the control the oxidants range from two-tenths to eight-tenths parts per million. The acids are the same in both lines.

Now, as to biological systems that have been tested by this means: One of the first that we have tried has been the erythrocyte. These were washed erythrocytes to remove the proteins so that any chemical changes occurring in the erythrocytes would be direct and not mediated via the plasma proteins. This, of course, has its disadvantages which I shall point out presently.

The first thing that we measured was for chemical changes in the

hemoglobin. There were none insofar as we could tell by spectrographic analysis. Very slight amounts of methemoglobin were formed, not much more than you would get if you ran room air through. There was considerable hemolysis which, of course, is the principal difficulty in this method of using washed erythrocytes.

One of the obvious things that would be suspected, which Dr. Kotin has mentioned and had observed previously, were changes in sulfhydryl compounds. They are very readily oxidizable; so, we studied the glutathione and the total SH compounds. Strangely enough we get slight decreases in glutathione with exposures of 15 to 20 minutes; at one hour they were back to normal. The total sulfhydryl decreases at the same time as the glutathione, and at one hour it is found increased. That of course, is consistent with the denaturization that is observed after that time. These changes are all relatively slight, considering the high concentrations at which we are operating, and are obviously of little import as biological indicators.

Next, we tried the uptake of radioactive phosphorus. You must recall that the system inside the red cell is chiefly set up for anaerobic glycolysis, and this is one of the changes that is noted, even in stored blood. So we thought that this might be a very sensitive indicator.

In dry air, just by exposing the red cells to clean air, we get a continuous decrease of P_{32} uptake at 15, 30, 45 and 60 minutes, compared to the unexposed cells. Now, when you expose to synthetic smog, you get the opposite, a decrease at the start and then an increase in uptake. I have no explanation for this. It happens on both the control and the test line, with both the irradiated and non-irradiated gases. This would indicate, therefore, that P_{32} uptake measurements would be of little help insofar as any specific effect of irradiation on the pollutants is concerned. However, it may have some indication or some use insofar as the general problem of air pollutants is concerned, because of the similarity in the reaction in both the control and the irradiated pollutants.

From this we moved to studying leukocytes, particularly polymorphonuclear leukocytes, prepared from guinea pigs. We can obtain these cells in yields of better than 90 percent from the material, something of the order of thirty million polys from each guinea pig, with a viability greater than 90 percent, as measured by vital staining techniques. The first very preliminary observations indicated a decreased viability as a result of exposure to synthetic air pollutants. This is not an immediate effect. It is one that gradually increases with time after exposure, and all of the exposures so far have been only on the test line for up to 15-minute intervals -- very short exposures. The change in viability is from a base of 90 to about 65 percent, and it continues to decrease. The viability of these cells normally is relatively short. Just on keeping them for four days or five days, they will,

of course, lose their ability to take up vital stains; but, so far in our measurements, while the unexposed cells have decreased only to 50 percent of their viability, the exposed cells have decreased to 6 percent.

The other information that has been obtained on a few brief exposures has been a decreased oxygen uptake of these white cells as measured in the Warburg apparatus. We have measured decreases ranging from 33 to 50 percent in the oxygen uptake of exposed cells compared with the controls handled in the same way but without the exposure. This is preliminary.

Similar viability studies have been started on *B. coli*. Here we have the question of strong sensitivity to acidity, especially when we are exposing the bacteria on plates; this involves some other apparatus that we are in the process now of testing. We have been primarily interested, since our finding of decreased oxygen uptake in the white cells, to test the oxygen uptake of *B. coli*, and so far the positive findings here consist simply of an early inhibition in the oxygen uptake when they are both treated the same. This means that in the first 15 - 30 minutes, of oxygen uptake, the capacity of the coli to respire has been somewhat impaired in comparison to controlled cells. We have not yet standardized this test as to the number of cells that are used, and this may make a considerable difference.

One of the most interesting observations that we made in July was an organoleptic one. We call it the "sniff" test. It does not seem to do our sinuses any good, for it seems to irritate the mucous membranes. There is a considerable difference, based on the observations of about 12 different individuals (anybody that I can get to come in to smell this material), between the low-humidity and the high humidity irradiated material. So, we tried to find a test object for this and settled upon the use of strips of rat and guinea pig intestine, suspended in a chamber, and studied under two conditions.

The first was a free suspension where the material could be washed with Ringer's solution and both the tonus and the contractions observed. We noticed a distinct difference between the specimens exposed to the dry-type of irradiated pollutants as compared with the moist irradiated pollutants, in spite of the fact that the oxidant values were relatively constant. When we use a superfusion technique, where there is a continuous flow of moisture over the tissue, the results are identical. The response of the tissue is identical. There is a relaxation effect very similar to that of isoamyl nitrite.

(A slide was shown.)

The next slide, however, shows the results of a technique in which we have had to allow free suspension. These do not show the nice clean-cut type of thing that we would like to demonstrate. The bottom portion is an exposure to compressed air alone. You will notice the contraction is somewhat rhythmic, and during this interval, which I think was one or two-minutes, we have had to remove the fluid, allow free suspension and then we see there is some contraction. After replacing the fluid there is a slight increase and then a decrease in the tonus. You get first an increased tonus by essentially the same strength of contractions.

Exposed to the dry smog for a period of one minute, then washed, you will notice that there is a tremendously increased tonus which continues for a considerable time in spite of the fact that we have washed the tissue.

When exposed to humid pollutants there is a diminution in contraction. Increased tonus remains, but you will notice that this tends to drop off. Now, this is the type of thing that we have also observed in all cases of exposure to either dry or wet smog by the superfusion technique. There is a diminution in contraction and finally, a diminution in tonus.

In summary: We have made observations of the biological reactions, both of cells and of tissues, since the toxic or irritating effects might demonstrate their activity either in the energy cycle or, on the other hand, in the neurogenic response for which the intestinal tissues were used; and, we have observed these changes.

The changes in the erythrocytes are not significantly different for the control and the test. So far the leukocytes and the B. coli studies involve only preliminary observations; but, we are somewhat heartened by the changes found. We cannot yet pinpoint the specific enzyme systems or co-factors that may be effected.

DR. HEIMANN (Chairman): To start the discussion, we will ask Mr. Manos to make some comments in connection with some work that he has done along some of the lines described by Dr. Gast.

MR. MANOS: We looked into past records for Los Angeles for a brief study of the relationship between humidity and eye irritation. Available to us were daily observations of eye irritation, oxidant level, humidity and other weather factors for the year 1955. The weather data and suggestions for its use were made available through Mr. Robert W. Schloemer, Assistant to the Director, Office of Climatology, United States Weather Bureau.

We used the oxidant level as an indicator of the level of air pollution, but not necessarily as the cause of eye irritation. We restricted the study to the six-month period, June through November 1955, and we further restricted it to only those days when the oxidant level was equal to or greater than five-tenths parts per million. This gave us 89 working days. We calculated the absolute humidity for each of these 89 days. Then we split up the 89 days into three almost equal groups: the first group contained those days with the lowest absolute humidity, the middle group contained those days with intermediate humidity, and the third group contained those days with the highest humidity. We found that for the group with the lowest humidity eye irritation occurred on 45 percent of the days, for the group with intermediate humidity eye irritation occurred on 33 percent of the days, and for the group with the highest humidity eye irritation occurred on 30 percent of the days.

DR. CHAMBERS: What were the absolute values of humidity for the "high", "low" and "intermediate" groups?

MR. MANOS: For the low group it was 1.4 to 4.7 grams per cubic foot of aqueous vapor, for the intermediate 4.8 to 5.5, and for the high it was 5.5 to 6.5. However, we were concentrating mainly on having an equal number of days in these three groups. There are 29, 30 and 30 days in the three groups.

We plotted the percent of days with eye irritation on the ordinate against the scale for humidity on the abscissa for each of the three groups. The trend is not statistically significant due to the small number of days, but it is in the same direction as the findings from Dr. Jast's laboratory work. The middle group is consistent in being between the two end groups. Our biggest fear in this type of finding is that a relationship like this, even though statistically significant, might be due to the variation in some weather factor, other than humidity, or to variations in the oxidant level. For that reason, we made a few tests to see whether we could explain this relationship through other weather factors or through variations in the oxidant level. For example,

we split up the 89 days into two groups, those with low wind speed and those with high wind speed and made this same breakdown according to humidity separately for the days with low wind speed and separately for the days with high wind speed. We get the same type of graph in each case. The frequency of days with eye irritation during low wind speed was 50 percent when there was low humidity, 40 percent when there was intermediate humidity, and 36 percent with high humidity. For days of high wind speed we get a similar trend except that we have a smaller percentage of days with eye irritation.

Then we repeated this same process by breaking up the 89 days into two groups according to the amount of cloud cover. The results show a similar trend except for inconsistency in the fact that the medium humidity group had a higher percentage of eye irritation than the low humidity group on days of little cloud cover. For days with a large amount of cloud cover we found that there was less eye irritation than on days with a small amount of cloud cover. This is consistent with past thinking.

If we repeat this complete analysis using relative humidity instead of absolute humidity, the graphs just do not make sense. They are no longer consistent at all. The graphs of the relative humidity data were not internally consistent. A higher percentage of eye irritation occurred in the low and high humidity groups than in the medium group. In examining the eye irritation for the three relative humidity groups on days of low and high wind velocity the inconsistency is accentuated. For example, in the medium humidity group a higher percentage of eye irritation occurred in the high wind velocity group than in the low wind velocity group. In the other two humidity groups exactly the reverse occurred. Similar inconsistencies were seen in other graphs of relative humidity data. I think one possible reason for this is that relative humidity is so dependent upon the temperature variation that we are not really studying humidity at all. We are really studying the temperature variations. Scatter diagrams of temperature with absolute and relative humidity for September 1955 indicate the close relationship of temperature with relative humidity and the lack of such a relationship with absolute humidity.

A final step, and this was really extending the data to the limit, was to see if we could get a consistent picture by studying the relation of eye irritation to the oxidant level and the absolute humidity simultaneously. We found that after considerable smoothing we could draw lines of equal percent with oxidant level on the ordinate and absolute humidity on the abscissa. The percent shown for any point in the body of the graph is the percent of days with eye irritation for those occasions when the oxidant level and the absolute humidity had the values indicated by the ordinate and abscissa of that point. If you draw any horizontal line on this graph and follow it from left

to right it tells you that if you take a constant value for the oxidant level, as your humidity goes up, the percent of eye irritation decreases. Similarly, if you draw any vertical line and follow it upward you see that for a constant value of humidity, as the oxidant level increases, the percent of eye irritation increases. It actually reaches 100 percent at the top of the graph. This was all based on only 89 days and considerable smoothing so that it cannot stand by itself. It is significant only when presented as supporting evidence for laboratory findings.

DR. HEIMANN: There are a few minutes left for discussion.

DR. SCHEEL: I would like to ask Dr. Gast if the intestinal loop recovers its irritability after exposure?

DR. GAST: After it is washed, it recovers.

DR. SCHEEL: I am particularly referring to the wet system.

DR. GAST: That depends on how long you exposed. For anything more than a few minutes of exposure they do not recover; but, you see, it has started in the middle one and it has started to recover. You see the bursts of activity. The tonus is roughly on the same line. All of these were done with transducers that were pretested and there was a recovery if you gave it time or wash out. Now, you see there is no wash on the wet one there at all and yet it came back. There are four washes on the dry one in order to get it back. That was done also to preserve the tissue.

DR. KOTIN: Did you measure the amount of NO that you had in your exposure chamber, and did you look for a peak of NO hemoglobin as well as methemoglobin. We found, as you did, that the methemoglobin formed was inconsequential; but, we found significant amounts of nitric oxide hemoglobin.

DR. GAST: I could not find any of either. I have made all of the preparations I could think of, and none were found in any of the exposed hemoglobin. Even the denatured hemoglobin did not have it. The protein is denatured without changing to NO hemoglobin. You see, we are not dealing with a lot of free NO or NO₂ at the point of exposure. Our NO₂ values are extremely low. All this is nitrated hydrocarbon.

DR. KOTIN: Did I understand you to say that you had a residence time of 40 to 70 minutes of the material in the chamber, or did you have a dynamic system?

DR. GAST: By "residence time" I am referring to the residence time in the irradiation chamber.

DR. CHAMBERS: One observation that might be pertinent to this question of the difference between wet and dry smogs was made at the U.S.C. The study was undertaken to determine the relationship between the humidity and aerosol formation and, ultimately, irritation in a synthetic system. So far, they have done it by setting up a forward angle light-scattering device which, theoretically, measures the amount of scatter formed by the aerosol-smog reaction. They found, in the initial phases of the work, that with up to about 20,000 parts per million of water content, the amount of aerosol formation seems to increase slightly. At about 20,000 p.p.m. it drops off very abruptly. There is a hooker in the observation, unfortunately. In the light of what is coming out here, it probably may turn out to be valid. But at the moment there is another explanation in these terms: The device has a fixed angle of scatter, and the pick-up is dependent on the scatter being in that direction. It may be that the drop-off above 20,000 p.p.m. is due to lack of formation of aerosol. On the other hand, it may well be that the drop-off is due to the fact that there is no variability in the angle of scatter and, as you know, the angle of scatter is a function of the diameter of the particles, so that it may simply reflect the difference in particle diameter. This point is being checked. I suspect, however, from the evidence in hand, that the high humidity does result in a substantial difference in the type of reaction that occurs.

DR. STOKINGER: There has been a lot of work done along these lines at Kettering with pyrolytic products of plastics which create a perfect smog, and there was a marked difference in toxicity in high humidities, along the lines of the information given here. Whether this is due to interaction of changed products is a different question; but, at least the overall data are in line with Dr. Gast's work.

DR. GAST: Unfortunately, I think there is one limitation inherent that we ought to keep in mind here in our work, as well as in what Dr. Chambers has described. There is no solid particulate here to help carry the load. What would happen in the presence of solid particulate is something else. The effect is going to be different depending on the relative attraction of the particulate particle for moisture as opposed to some of the other materials.

DR. HEIMANN: The next speaker will be Dr. Pace, who will discuss his work on tissue culture.

DR. DONALD M. PACE: In our laboratories at the University of Nebraska we are investigating the feasibility of using tissue cells in vitro as a means of evaluating the toxicity of air pollution. We have studied the effects of two substances that appear as constituents of polluted air; these are sulphur dioxide and nitrogen dioxide.

Most of our difficulty, at first, was obtaining the types of cells with which we could work; this is not always easy. There are certain strains already established; the term strain is a poor one to use here -- we should call them clones. So far we have used three types (or clones) of cells -- Strain L, which is a clone of fibroblast cells; an epithelial cell, the HeLa carcinoma; and, a normal liver cell clone.

We are studying the effects of the gases on (1) growth itself, whether inhibited or accelerated; (2) metabolism by the Warburg method; and, (3) morphological changes that may take place, by means of lapse-time photography.

(A slide was shown.)

Ordinarily, in the experimental setup, we use D-3.5 Carrel flasks and, for stock cultures, Earle's T-60 flasks. The first two are T-flasks. They were devised by Dr. Earle of the National Cancer Institute; T-60 simply means that there is a 60 square centimeter surface. The other type is a T-15; a Porter flask, and a Carrel flask are also shown. The Carrel flasks are used because they are small and many of them may be handled at one time. However, it looks as though we are going to use T-60 flasks entirely for this work. I will mention more about this later.

When the cultures were set up, one ml. of medium with the cells in it was added to each flask. The culture medium for the first part of our work consisted of our usual "witches' brew" made with horse serum, embryo extract and a balanced salt solution. This consists of 4 parts horse serum, 4 parts saline and 2 parts embryo extract.

In the first tests, we used rather high gas concentrations. Consequently, we had trouble with pH changes and in many instances, if not all, it was the high hydrogen ion concentration that killed the cells, rather than the SO_2 or NO_2 . In the lower concentrations used, we were not troubled much with this.

The concentrations used in these experiments are high compared to those that are found in air. The culture flasks containing the cells were flushed out with a chosen concentration of SO_2 or NO_2 in air- CO_2 mixture. We used 5% CO_2 in air. After two days' exposure, the nutrient media were replaced with fresh media; the cultures were then reflushed with the gas mixture. In other words, we stoppered them

after flushing with the mixture, put them aside for two days, and then noted what had happened during that time; this was repeated every two days until the end of the experiment.

The next few slides illustrate the results of some of these earlier tests. I am sorry I do not have slides for some of the later tests. At any rate, we are going to change our approach to such an extent that these results may not mean much, except that some of them indicate rather interesting possibilities.

(A slide was shown.)

Here are some of the results obtained by exposing Strain L cells to different concentrations of SO_2 . This graph is typical for the results we obtained. Several tests were run using 0.05%, 0.1% and 0.2% SO_2 . As shown in the graph, the control is running along as we expected. The cells in the 0.05% SO_2 increased in numbers to a greater extent than those in the control cultures, but those in higher concentrations did not do so well. Next slide, please.

(A slide was shown.)

This figure is a compilation of the results obtained with the liver cell strain which I mentioned previously. When we exposed the liver cells to SO_2 in these concentrations, we found that with 0.05% and 0.1% SO_2 , growth was approximately the same as in the control solution, and slightly less in the 0.2% SO_2 .

(A slide was shown)

This figure represents growth of HeLa cells. They grow much more slowly and take a much greater time to cover the surface of the flask than it takes for the Strain L or the liver cells. In the HeLa cultures at the end of 10 days, in either 0.05%, 0.1%, or 0.2% SO_2 , the cells were beginning to die; apparently no proliferation. There were fewer cells than we had started with, whereas in the control they were quite normal.

(A slide was shown.)

This photograph is somewhat out of focus. We do this purposely so that we can see the outlines of the cells. This is a normal culture of Strain L cells after about 10 days.

(A slide was shown.)

This is a photo of Strain L cells exposed to 0.2% SO_2 .

(A slide was shown.)

The next photograph was taken of Strain L cells in 0.1%. There are many more cells than in the previous cultures. The next slide is a photograph of the same strain in 0.05%.

(A slide was shown.)

The cells are so numerous that they are about to become necrotic. The culture is very dense as compared to those in higher concentrations of SO_2 .

(A slide was shown.)

These are the HeLa cells. They are very difficult to see or to photograph unless they are out of focus. This is a photograph of a control culture. The next slide is a photo of HeLa cells in 0.2% SO_2 at the end of ten days.

(A slide was shown.)

This is a culture exposed to 0.1% SO_2 .

(A slide was shown.)

Note the odd shapes shown by these cells. The next slide illustrates the effect of 0.05% SO_2 on HeLa cells.

(A slide was shown.)

The results obtained with NO_2 were practically the same as those obtained with sulphur dioxide. I do not have illustrations of these experiments. We used concentrations of between 0.5% and 0.05% NO_2 and noted the effects of these concentrations on the growth of the cells.

After noting the results obtained with these higher concentrations of SO_2 and NO_2 we exposed the cells to the lowest concentration in which we could get growth similar to normal growth. We then studied the effects over a long period of time. A "long period of time" in this case was two months. During this time we changed our culture medium to a synthetic medium made up of 68 different substances plus 10% horse serum. We did without the embryo extract and it looks as though in the very near future we will be able to carry on this work in a completely synthetic medium. The cells do very nicely in it. However, we want to play safe and we want to make sure that they will grow indefinitely in these fluids because there have been synthetic media devised in which the cells would do well for several months and then die. We want a synthetic medium in which the cells will grow

indefinitely. When we did carry them at these low concentrations of SO_2 over a number of weeks, we found that the cells exposed to 0.1% and 0.2% SO_2 seemed to become adjusted to their environment and did much better at the end of eight weeks than at the beginning of the experiment.

We are now proposing to carry out our experiments in a different manner. We are going to set up T-60 flasks in such a way that continuous flow of gas will pass through them. If possible, we want these gases exposed only to glass. This is difficult when you consider the number of attachments necessary when two or three hundred T-flasks are used. I am attending this meeting chiefly to get suggestions that some of you who are well acquainted with this field, might be able to offer.

DR. HEIMANN: Thank you. We have just about five minutes for discussion.

DR. EDWARD J. FAIRCHILD: Is your criterion for growth based primarily on division and multiplication of the cell?

DR. PACE: Yes. If the cell divides very rapidly, it does tend to remain smaller. We do make measurements.

DR. FAIRCHILD: The main thing I was wondering about was, did you notice more aberration in cell structure? Was that taken into account or was it just the number of cells that concerned you?

DR. PACE: In these early tests we were just trying to count the cells. We do have some lapse-time motion pictures; but, there are many difficulties, especially in these higher concentrations. For example, when we started taking lapse-time pictures of cells in nitrogen dioxide, everything seemed to be working fine so we left the mechanism to run all night. In the morning we found that we had used a whole reel of expensive film, taking pictures of dead cells during the night. Such difficulties may become expensive. It is our thought to get lapse-time movies to show morphological changes along with these others. Up to this time we have made very few metabolic tests.

DR. KOTIN: Did you expose your nutrient media, especially your synthetic media, to the pollutants prior to feeding them to your culture, since some of these amino acids have a high affinity for the gases? There could be alterations of the media by a response to the pollutants. We found a significant difference in pre-treating the nutrient media on the basis, not of the effect of the tissue culture per se, but on the basis of the fact that you alter and denature many of the proteins in your nutrient media. I wondered

if it might not be an additional control you might use. I have found it to be very helpful.

DR. PACE: We have not done so, but we will. We have noted that the color of the medium itself turns from a light to a dark yellow.

DR. STOKINGER: Do you think the means of introducing your gas represents a fairly level concentration of gas, or does it allow peaks?

DR. PACE: That is one of the reasons I did not make graphs of our nitrogen dioxide tests. We are changing the whole setup, because we realize that we are not getting the true picture. We now realize that we must change our method of introducing the gases to one of continuous flow. Perhaps, if we use a layer of fluid sufficiently thin, it may be possible to gas it properly. We are limited in this approach when you consider the numbers of cultures that must be used in order that the results will be valid. We must not use too small a quantity of fluid or it would be necessary to change it too often.

DR. HEIMANN: Thank you very much, Dr. Pace. May I remind you again, as Dr. Fisher said earlier, these experimental results that are being discussed here this morning and the data presented are preliminary.

The next speaker this morning is Dr. Tebbens. Before Dr. Tebbens starts, may I say that he is going to talk mostly from the point of view of the chemist. However, we thought that his work would be significant for us, so in this case we have made this exception. We think that what he is going to say is of great importance to those of us working in the field of air pollution toxicology.

DR. TEBBENS: This is non-biological completely, I assure you. My interest in what I shall call hydrocarbon synthesis in combustion started several years ago when a graduate student, in separating components of smog from air of Berkeley, found a fluorescent material which chromatographed in a way that was reproducible. Since that time we have undertaken experiments with fuels, burning these fuels incompletely, with a smoky flame, picking up the smoky combustion by-products by filtration, separating from that smoke the ether-soluble portion, and then separating that ether-soluble material into a series of chemical products. The immediate interesting part, I think, which can be applied biologically,

is the finding that in these high molecular weight hydrocarbons that are produced, there is a series of polynuclear aromatic neutral compounds, and from them evidently a series of oxygenated compounds as well, phenols and some organic acids.

In all, we think, we have separated as many as 90 different chemicals of high molecular weight from the incomplete combustion of as simple a fuel as methane; starting with one carbon atom, if you will. In the byproducts we find compounds of molecular weight running up as high as almost 300, and perhaps there are even heavier ones. Included in this group are benzpyrene and dibenzanthracene, both of which have been accused of being carcinogens. Also, there are several other components of high molecular weight which, theoretically, I understand, may be carcinogenic.

We chose a series of simple gaseous fuels to start with; methane was one. It actually was natural gas. Then we tried acetylene, butane, butadiene and butene. Finally, we also used propane. All of these fuels, when burned with a smoky flame, will produce these by-products. We assume that there must be some typical chemical reaction involving free radicals that leads to these end products which are so similar, no matter what kind of fuel we use. Our original intent was to start with the simple fuels which we thought we would work out in a few weeks, and then go to liquid fuels, and finally to solid fuels, to see what were the similar products and the by-products in the combustion of similar compounds. We are still working on these six gaseous fuels because the problems of microanalysis of the components is so very time-consuming.

Recently we burned a few liquid fuels in a completely different kind of burning equipment than we had used for the gases, and found that some of these liquid fuels produced less of these by-products than we anticipated. In other words, we can find the same "arenes", as we call them, or polynuclears, from burning hexane as we can from burning propane, but they seem to be in smaller quantity in the former. The reasons for this we have not worked out yet. We are retooling now to enable us to burn these liquid fuels in the same kind of combustion apparatus in which we burned the gaseous fuels.

In one instance we took the incinerator stack effluent from the combustion of paper, cardboard, and tree trimmings, and other rubbish as may be found in Los Angeles and other places, and from the very small amount of smoke from an experimental combustion of this solid fuel, found, again, the same series of chemical compounds. In one instance in which we examined the gas from an automobile exhaust, we found the same sort of thing.

In the air samples that we take occasionally in Berkeley, separated by the same methods, we find the same series of compounds. This is not typical of Berkeley alone, I am sure. Kotin reports it from Los Angeles, the British from London, and the Russians report it from Moscow. Wherever these high molecular weight hydrocarbons are looked for in the air, if they are looked for hard enough, they are found. Evidently this all relates to incomplete combustion. It does not necessarily relate to the combustion of a specific fuel, as far as we can guess. It relates simply to the process of incomplete combustion itself as a chemical process.

There are some additional implications probably involved in these findings; for example, there must be many other kinds of chemical entities associated with the by-products of incomplete combustion, but of lower molecular weight. If you can start out with methane and build high molecular weight hydrocarbons of up to 300, at least, the chances are good that you can build hydrocarbons with molecular weights half of that and a quarter of that. In other words, there must be a whole series of such compounds produced. This has been partially confirmed by others. For example, I find that recently workers at Battelle Memorial Institute, in separating out material from combustion of solid materials in an incinerator, found alcohols, acids, and saturated and unsaturated hydrocarbons in the liquid chemical ranges.

I might point out that on complete combustion of these fuels none of these products are found in the flue gases. One has to have something like smoky combustion to get this synthesis reaction. On a beautifully clear day in Berkeley we do not find them in the air. On a smoggy day we do. We are not certain of their concentration in the air. Quantitative studies have only just begun. From the quantitative point of view I can say only that from an air sample, about ten percent, for example, of the filterable material is extractable in ether. And this can be divided into these many compounds, at least 90. Apparently, at least half of the weight of this extractable material is of the neutral hydrocarbons. The other half is distributed between phenolic and carboxylic compounds, which is different from what we find from burning some of the straight gaseous fuels.

When we burn straight gaseous fuels such as propane we find up to 20 percent by weight of the filterable effluent is extractable. It contains a higher proportion of the neutral compounds than occurs in free outdoor air. This will amount to about 90 percent in most of the cases in which we have made the separations.

If there are any questions I can answer, I will be glad to do so.

DR. HEIMANN: The relationship of this work to biological work is important; and, I wonder if anybody would like to make any comment. Dr. Kotin, would you like to comment?

DR. KOTIN: I would like to ask Dr. Tebbens if he attempted to determine what percentage of these "arenes" are in their stable form and what percentage show either oxidation, or nitration, etc.

DR. TEBBENS: We find practically no nitrogenous compounds. From a stack effluent about 90 percent, in most cases, is in the form of "arenes", the neutral hydrocarbons. The other ten percent is split between phenolic and carboxylic compounds.

DR. KOTIN: But, in the outdoor air?

DR. TEBBENS: Outdoors, it will be closer to 50 percent "arenes", as has been shown in the very few samples that we have taken so far.

DR. CHAMBERS: There appears to be a remarkable correlation between the ether soluble component of the particulate matter collected and the degree of eye irritation reported in Los Angeles. In fact, the timewise correlation with that one thing, on the basis of this year's study, seems to be much better than with any other single type of measurement we have been able to make. You mentioned that the ether solubles in the San Francisco area, -- I assume during smog period -- amount to about 10 percent. Is that correct?

DR. TEBBENS: That is right.

DR. CHAMBERS: In Los Angeles, during heavy smogs we have had as high as 30 to 32 percent of ether-soluble fraction of the particulate, and from the last January 4th and 5th episode of smog in London, we had a series of samples which were phenomenal in weight; and, they actually showed 51 percent ether solubles. All of these are presumably related to the type of thing you are talking about.

DR. TEBBENS: It sounds very much like it to me. I am certainly convinced in my own mind that incomplete combustion has something to do with this whole phenomenon, whether biological or physiological.

DR. CHAMBERS: There is one other application of this that is of great concern to us in Los Angeles where we burn hundreds of millions of cubic feet of natural gas per day in domestic hot water heaters and domestic furnaces, many of which are not constructed for efficient combustion. It raises a serious question as to whether we have not been ignoring the improper combustion of natural gas as the source of some of our trouble.

DR. HEIMANN: Are there any other comments or questions? Since there are not, we will call upon our next speaker.

Dr. Phair has been working on air pollution effects here in Cincinnati for some time, and will tell us about some of his methods and results.

DR. PHAIR: We have been interested in the field of Air Pollution for some time. As a matter of fact, our interest in this area began long before I came to Cincinnati, in that the Kettering Laboratory has been conducting various types of studies for many years, measuring different atmospheres both in industrial plants and in various communities. When I came, we were concerned about the fact, and I think that this is very important, that the engineers and chemists had far outrun the clinicians, and that there were able to measure fine differences of a great many different materials. Then they come to the physician and say, "Look, we can measure this; there are significant changes; therefore, something must be happening to man." Yet, when we turn to man, we have no way of objectively measuring his reaction to variations in these pollutants.

So it seemed wise, back in 1951, to give some consideration to setting up a project which would permit us to obtain some information about the "Reactions" of man to variations in these pollutants in the air. I say "Reactions" advisedly, for I am restricting my presentation entirely to acute reactions. We have not been interested in the chronic reactions of man, which is an entirely different field of study and would require different techniques and different approaches. As yet, I have no idea how we might tackle that problem.

However, using our past experience as a base line, we began by attempting to draw up a project which would seem satisfactory, and in this phase, we encountered many difficulties. Finally, we secured support from the Public Health Service. With these funds, we set up an advisory committee made up of certain people who had a nation-wide reputation in certain areas of preventive medicine, and we brought them together with the idea of securing their advice in helping to design our project. We had a meeting that, in many ways, was very similar to this meeting and others that Dr. Fisher has arranged, in which we tried to get together and determine the things which we might try to do.

The first difficulty that we encountered, was our inability to distinguish between areas of high and of low air pollution. In other words, we would have some areas that we might say are badly polluted; yet at times, when the prevailing wind is different, would be just as good as our best areas, and vice-versa.

The second difficulty was our inability to utilize cardio-respiratory patients unless they could be tested objectively at regular intervals. It does not suffice to follow such patients only with a history, or to record subjective sensations; one must test them objectively as well, to get any useful information.

The third difficulty that we found was that of our inability to use neighborhood atmospheric measurements as an index of the dosage of pollution which the patient was receiving. In other words, what our patient breathed in his own home was something entirely different to that which would be measured outdoors, even two or three blocks away. These three difficulties limited our approach and we finally decided to study a small group of cardio-respiratory patients, each confined by his disease to the neighborhood of his own home. Each man or woman would constitute an individual experiment, and each would be observed periodically. At the same time, we would make measurements of the atmosphere in their own immediate environment.

For these reasons, we had to develop new methods of measurement. For the patient, we recorded the subjective reactions and the objective reactions. For the environment, we wanted to take records of temperature and humidity, and we wanted to measure the amount of contamination of the air. This meant that we had to devise techniques which could be used in the room of each patient.

A stained wooden cabinet, approximately 18 inches square and 30 inches high, containing one Hemeon AISI smoke sampler and one thermohygrograph, was placed in the home of each patient. Temperature and humidity are recorded by a thermohygrograph placed in the bottom compartment; the humidity is recorded by the contractions and expansion of a bundle of hairs. The temperature is recorded with a bi-metallic unit. In the section above the thermohygrograph is placed a Hemeon AISI sequence sampler which gives hourly readings of the amount of particulate matter that was in the air of the home. In order that we should not disturb the patients too much, it was necessary to sound-proof these cabinets. It was also necessary that the cabinets should not interfere with the ordinary life and habits of the patient, and should fit into his room and match the furniture fairly well.

The amount of smoke present in the air is determined by comparing the density of the smoke stain produced by the AISI (Hemeon) sampler with that of unsoiled filter paper. For this purpose, we use a Photovolt photometer which measures the transmission of light through the smoke spot on the filter paper. The value obtained is compared with the value of (100 percent) transmission through the unsoiled filter paper adjacent to the smoke spot. In this method, we have to allow for the variation in the thickness of the paper; hence,

it is not possible to get a steady base line of 100 percent if an automatic Brown Recorder is used. We have found it more convenient to use the older hand-operated photometer which can be adjusted to 100 percent before and after measuring each smoke spot.

For the first survey, we measured the inspiratory and expiratory pressures of the patients by means of an adapted aviation altimeter. Timed vital capacity tests were also done, measuring the amount of air expired during the first, second, and third seconds of a forced expiration, and total vital capacity of the patient, using the Gaensler vitalometer. We also did an oximeter measurement of the oxygenation of the blood.

In the first survey, we saw each patient once a week and did three tests on the patient and serviced the machine each time. These tests of inspiratory and expiratory pressure and the timed vital capacities were done before and after the administration of a bronchodilator. For the second survey, we discarded the oximeter because the subjects were cardio-respiratory patients and could not be put under exercise stress. Under resting conditions, the oxygenation of the arterial blood shows very little fall unless the patient is in great need of oxygen. Under the conditions of our survey, it was not possible to show large enough variations in the oximeter readings to be truly significant whilst the patients remained at rest.

The Gaensler vitalometer was used in the first survey and proved heavy and difficult to handle. The nurses complained about the difficulty of carrying this heavy equipment up to the third or fourth floor of some of the tenement houses or housing units in which our patients lived. Therefore, we combined the McKesson-Scott bellows type of vital capacity meter with the timing mechanism of the Gaensler vitalometer and assembled it in a little box which is about the size of a portable typewriter. The nurses were able to carry this much more easily.

In the third survey, which is under way at the present time, we began the measurement not only of particulate matter but also of sulphur dioxide. A small sequence sampler was designed and constructed in our laboratory. Eight of these units have been used in the homes of patients. The sampler consists of a very small, almost noiseless, diaphragm type of pump. A telechron clock turns a cam mechanism which actuates a series of microswitches to complete the circuit through electrically operated solenoid valves. In this way, we can take six-hour samples of sulphur dioxide in the homes of patients. Twelve solenoids and twelve bubbler tubes are used so that the equipment can run for a total of three days before servicing is required. The entire sequence sampler is housed in a soundproof box of similar color and

construction to that which houses the neon smoke sampler and the thermohygrograph. Since this sequence sampler was designed and built, an even smaller sequence sampler, which may be even more suitable for use within the homes of patients, has been developed at the Kettering Laboratory.

In all these tests, we were forced to develop methods, not only of measuring the patient, but the patient's environment, and do it in a way that the patient would accept. We found a great deal of difficulty in securing the cooperation of these people. They liked visits up to a certain point; but, when one begins to come into their homes three times a week they become somewhat more concerned about it, particularly if one subjects them to various kinds of lung function tests.

The first survey was done in the spring of 1955. At this time, we measured patients objectively in three ways: inspiratory and expiratory pressures, timed vital capacity, and by the oximeter. These tests were done before and after a broncho-dilator. Measurements of the external environment included only temperature, humidity, and particulate matter measured as smoke. The present status of that particular survey is that most of the material has been assembled and punched on cards. We choose, arbitrarily, certain lag periods between which we would expect the environmental insult to affect the patient and for his reactions to occur. We are now trying to write a program which will permit us to use the 704 electronic computer of the General Electric Company in doing a multiple regression analysis. It has been found that it is a very difficult thing to handle the data for such an analysis and to get the necessary program prepared. Possibly we should have attempted some other method or some other approach to solving these equations. At the present time, having committed ourselves to this type of statistical analysis, we will have to follow through on it until we come out with the necessary program. Once that has been achieved, it is understood that the results should be obtained fairly readily.

Superficially, we do not believe that we are going to be able to show much correlation between the reactions of patients measured in our original survey and variations in the temperature, humidity, and particulate matter. It is possible that some correlation may show up after the analysis is complete. The first survey was an attempt at the design and organization of the air pollution survey and we ran into a lot of logistic problems. We had to get a trained team organized. Graduate nurses were used in this first study, and we found that they were not so good as practical nurses whom we have used in the subsequent two studies; and, there were a number of other problems. Although the

material which we have backlogged will be useful in developing our mathematical handling of such epidemiological studies of air pollution, one must not be too optimistic about the results to be obtained.

Immediately after the spring study of 1955 had been completed, we began making plans for the second study to begin in the fall of that year. We thought that some of the difficulties which we had encountered or our failure to show correlation, might have arisen out of the fact that we came from bad weather to good weather. In other words, the patients might have become fairly well adapted to smoggy conditions before the first study had commenced and were less likely to show significant reactions as they moved from the bad weather of winter into the better weather of spring. So, we thought that it was worthwhile to start right over again the following September and move from good weather to bad weather. Once again, we recorded the subjective reactions of the patients and again we tried to do objective tests. Inspiratory and expiratory pressures and timed vital capacity tests, using the modified vitalometer, were done twice a week. The tests were first done without the use of a broncho-dilator and then after administration of "Aerolone," the tests were repeated. The oximeter tests were not used in this second survey. No change was made in the method of measurement of the external environment in respect to temperature, smoke, and humidity.

All this material is backlogged and is awaiting the completion of the program for the IBM 704 machine so that the analysis can be completed. After the end of the second survey, we spent some eight or nine months attempting to improve our patient testing. We were concerned with the fact that the tests of inspiratory and expiratory pressures and of timed vital capacity require patient cooperation. The only test used to date which did not require patient cooperation had been the oximeter test in the first survey, but this had been discarded because of inaccuracies inherent in the test itself. Therefore, we reinvestigated lung function tests done in other laboratories and tried to develop a mechanism which would permit measurement of carbon monoxide uptake. Such tests in the past required the use of very cumbersome apparatus, but we were able to modify this test and to put the essentials of the apparatus into a small box-bag which was entirely portable. The equipment, as finally developed, could be carried quite easily by the practical nurse into the patient's home, and the patient found no difficulty in tolerating these tests being performed three times a week.

As finally developed, our box-bag consists of a one foot cube box constructed of transparent lucite. Within the bag are two meteorological balloons of 10 liters capacity. Two three-way valves are placed on the top of the box so that the patient can either

breathe air outside the bags but still within the fixed volume of the box, or on a closed circuit from bag to bag. On the top of the box is a small rubber bellows open to the box itself, so that we are able to determine the rate and amplitude of breathing as indicated by an arrow attached to the bellows and the scale.

In our technique of carbon monoxide diffusion, the patient breathes in and out of the box with his nose clipped until we get a constant rate and constant amplitude of respiration as indicated by our bellows and arrow. The valves are then closed, and the patient begins breathing through a one-way mouthpiece valve from one bag into the second bag. At the beginning of the test, the inspiratory bag contains 10 liters of accurately measured carbon monoxide (0.1 percent), helium (12 percent), and room air. During the course of breathing, the patient will respire the 10 liters of mixture from the inspire bag, transferring it through his lungs and expiring into the second or expirate bag. By taking the difference of the concentrations between the two bags, one can tell how much carbon monoxide has been taken up by the blood, and how much the helium has been diluted by the air originally in the lungs. The total time of test and the number of respirations are counted. In this way, we are able to arrive at a figure of carbon monoxide uptake and also to arrive at an assessment of the functional residual capacity of the patient.

In our third survey, this carbon monoxide uptake test was used as well as inspiratory and expiratory pressures and the timed vital capacity techniques. Our patients were followed even more closely and the nurses visited the patients three times a week. This has introduced additional logistic problems and we have had to reduce our number of patients from 30 in the first two surveys to 10 in the third survey. Thus, we have added two new tests in the third survey, firstly, the carbon monoxide diffusion technique, and secondly, the measurement of sulphur dioxide as well as particulate matter in the home of each of the patients. This particular study is just being finished and the machines are coming out of the homes of the patients this week (December 18).

It seems that this carbon monoxide uptake test appears to be quite sensitive, and during the one smog period which we had in Cincinnati in October 1956, it looked as though carbon monoxide uptake was reduced in our patients while the functional residual capacity ran along unchanged. But these impressions cannot be confirmed until a complete statistical analysis is done. We are

hopeful that with this new technique of carbon monoxide uptake we will have a truly objective method of measuring the reaction of the patients to air pollutants. We proposed to go forward with this type of study and test under controlled conditions in the laboratory the reactions of normal subjects and possibly of cardio-respiratory cripples, to some of the more common air pollutants.

DR. HEIMANN: Thank you very much, Doctor Phair. May I suggest that we take a break for five minutes now, and anybody who would like to ask Dr. Phair some questions can do so personally. I am sure he will be happy to answer.

(Thereupon, a short recess was taken.)

DR. HEIMANN: Again our apologies for trying to push so much into such a short time and leaving so little time for discussion. It now gets worse. There is not even any time allowed on the program for discussion for the next six papers. They were put in because we thought that they were important enough that you would like to hear them. We have asked each one of the representatives of the Occupational Health Field Headquarters to talk for, at most ten minutes, presenting his findings. Should you find it desirable to discuss some phases of this with any of them, I am sure that they will be available tomorrow afternoon.

The first speaker will be Dr. Saltzman, who will talk about the kinetics of ozone and hydrocarbon reactions.

DR. SALTZMAN: Synthetic smog, duplicating the eye-irritating and plant-damaging characteristics of Los Angeles smog, has been produced by the reaction of ozone with olefins. However, the exact processes and active products in such reactions are not clear, and were therefore investigated. Many simultaneous and consecutive processes occur; thus the time factor is very important.

If the usual kinetic equations are applied to bimolecular processes, we can readily show that the half-life varies inversely with the concentration, as shown in the following table:

RELATIVE HALF-LIVES

<u>0.1 P.P.M.</u>	<u>1000 P.P.M.</u>	<u>10%</u>
1 hour	0.36 sec.	0.0036 sec.
6.9 days	1 min.	0.60 sec.
1.9 years	100 min.	1 min.

Thus, if we take for study under ordinary concentrations (10%) a process which happens to have a half life of 1 minute, which is relatively fast, such a process would be hopelessly slow at smog concentrations (0.1 p.p.m.), with a half life of 1.9 years. Similarly, if we selected a process with a half life of 1 minute at 1000 p.p.m. (typical of low concentration kinetic studies), such a process would be very slow (half life 6.9 days) at smog concentrations. The processes we are concerned with (half-life of say 1 hour at smog concentrations) would be extremely rapid at ordinary concentrations, with a half life of milliseconds. These calculations indicate that very little information presently available is applicable to the study of smog reactions, and that we must be prepared to deal with extremely reactive and unstable materials with which we may now be totally unfamiliar.

The reaction of ozone with hexene-1, taken as typical, was investigated at intermediate concentrations and at age $1\frac{1}{2}$ seconds with a mass spectrometer specially designed for the study of fast reactions and free radicals, made available by the Applied Physics Laboratory, The Johns Hopkins University. It was investigated at low concentrations and age 10 to 20 minutes by two chemical iodimetric methods.

A tentative interpretation of the mass spectrometric analysis was made for about 800 p.p.m. of hexene-1 and for the same after generation of synthetic smog by addition of about 1500 p.p.m. of ozone and aging 1.4 seconds. These data indicated the following:

1. The reaction is much faster, and hence more important in smog processes, than previously thought. This is implied by the complete disappearance in the short reaction time of the lines for hexene, and for four and five-carbon hydrocarbon fragments.
2. Study of the oxygenated fragments implies that not only are the double bonded carbons in the hexene molecule attacked by oxygen, but also the adjacent (alpha) carbon.
3. Many simultaneous reactions appear to occur, producing a variety of products, such as four and five carbon aldehydes, four carbon hydroperoxide and peracid, carbon dioxide, formaldehyde, and methanol. The ozonide was not observed, nor could all the products be derived as fragments of the ozonide.
4. There is an indication of the presence of free radicals, such as CH_3O_2 . The ionizing voltage of the mass spectrometer was reduced from the 50 volts used to much lower values, in order to reduce fragmentation of the molecules in the instrument. The lines persisted, implying that these fragments actually existed in the sample, rather than being made in the spectrometer.

The same reaction was also studied chemically at a concentration of 3.5 p.p.m. hexene-1 and -3 to 28 p.p.m. ozone. Oxidants were determined by simultaneous analysis of the fresh mixture, and the mixture after 10 and 20 minutes aging in a flowing system. A neutral and an alkaline iodide procedure were used, the latter being corrected by a stoichiometric factor. Both methods agreed for the fresh mixtures (unreacted ozone), but diverged for the aged mixtures. The alkaline method showed a much greater rate of oxidant consumption. This is presently interpreted as indicating that some reaction products continue to liberate iodine from the neutral, but not from the alkaline reagents. Such a technique may be a means of differentiating smog oxidants.

In conclusion, there is strong evidence that the reaction of ozone with hexene-1 to produce synthetic smog is a free radical reaction. This is suggested by the extreme rapidity of the reaction, by the non-integral stoichiometry, and by the variety of products formed. The free radicals suggest an analogy between the biological effects of oxidative smog and X-rays and other ionizing radiation, since the latter are also thought to act through the mechanism of free radicals (of OH and HO_2). If we replace the H by organic groups designated by R, we get the analogous free radicals RO and RO_2 , which are the very ones likely to be present in oxidative smog.

DR. HEIMANN: Thank you Dr. Saltzman. The next speaker is Mr. Floyd who will present the screening toxicity studies on organic peroxides and hydroperoxides.

MR. E. P. FLOYD: Organic peroxides are likely constituents of certain types of (oxidizing) smog. For this reason, tests are being conducted in our laboratory to determine the toxicity of these compounds on small mammals. This has been under way only a few months. The specific compounds were selected on the basis of their availability, their known use in industry, and the feasibility of their use in the laboratory for experimental tests. I will show the types of organic peroxides from which we must select compounds for certain experiments. First, hydrogen peroxide is the basic structure for these, the organic hydroperoxides and the organic peroxides in general. (H-O-O-H , R-O-O-H , R-O-O-R .) In addition to this, of course, these may be mixed; (R-O-O-R') probably, we will have to synthesize the compounds of that nature.

The particular ones which we have selected for our preliminary studies include t-butyl hydroperoxide, di-t-butyl peroxide, methyl ethyl ketone peroxide and cumene hydroperoxide. A number of tests have been made on other compounds, but they are only very preliminary ones. The LD-50 on rats by intraperitoneal injection has already been determined on these compounds. These results, along with similar tests by gavage, indicate rather extreme variations in toxicities of organic peroxides. A particularly interesting variation is the finding that the hydroperoxide is far more toxic than its equivalent peroxide, approximately 50 times the toxicity, as a matter of fact.

Tests on rabbits show that each of the peroxides mentioned is a primary irritant at full strength and some of them are mild irritants when diluted as much as 50 times. These particular ones, incidentally, are cumene hydroperoxide and methyl ethyl ketone peroxide, which show the primary irritation at that great dilution. The diluents, dimethyl-phthalate and propylene glycol showed very slight or no irritation.

Other tests which we are now performing with these compounds include using the eye and inhalation routes, and also repeated doses of 20 percent of the established LD-50. This work is done by intraperitoneal injection and the doses are repeated on Monday, Wednesday and Friday of each week. On these four chemicals mentioned, this was done for seven weeks and the animals were examined. There were very few deaths, though the LD-50 was exceeded by quite a large amount. Sensitization of guinea pigs is also being studied and the modes of action of these compounds are under investigation. For example, blood of exposed animals is being tested for possible methemoglobin formation, and the serum is being checked for any changes in protein.

DR. HEIMANN: The next speaker will be Dr. Svirbely, on the toxicity of hydrogen peroxide-ozone mixtures.

DR. J. L. SVIRBELY: Tests for possible ozone contaminants especially free radicals, made in animals in this laboratory, and with the mass spectrometer elsewhere failed to reveal significant amounts by these techniques. The possibility of free radicals and/or H_2O_2 has not been ruled out since the sensitivity of the instrument was estimated at 1 to 10 ppm (per cent by volume) for H_2O_2 at 200 ppm and for HO_2 radical at 50 ppm. Since H_2O_2 may be associated with O_3 and its reaction products with water and organic substances, it was considered desirable to make a study of the toxicity of these two substances in admixture. The study at the present time has been limited to the determination of acute and subacute effects of some possible air contaminants and involved single exposures at relatively high levels.

Generally, 10 male mice and 5 male rats were exposed simultaneously in an 18.5 liter glass exposure chamber, to non-lethal, easily tolerated concentrations of O_3 and H_2O_2 separately and in combination, in an effort to determine whether an enhancement in toxicity occurred when these two substances were mixed. No deaths or outward toxic signs were observed when the animals were exposed for a period of 4 hours to chamber concentrations of 1.1 ppm O_3 or 16.1 ppm H_2O_2 , respectively. In admixture, with the ozone level maintained around 1 ppm, the mortality in a 24-hour period following a 4 hour exposure with varying concentrations of H_2O_2 , were:

H_2O_2 ppm	Mice	Rats
16.6	3/10	1/5
7.8	2/10	2/5
3.3	3/10	0/5
1.5	0/10	0/5

In another study, by varying the ozone concentration and maintaining the H_2O_2 concentration from 7.5 to 10.2 ppm, the LD_{50} values for ozone calculated for a 24 hour period (4 hour exposure) were:

4.8 ppm (range of 3.4 to 6.8 ppm) for mice
3.4 ppm (range of 2.5 to 5.0 ppm) for rats

Hamsters were found to be resistant in these studies as previously reported for ozone. The mortality found in a 24-hour period (4-hour exposure) was:

Concentration (ppm)		Mortality	
H_2O_2	O_3	Mice	Hamsters
24.8	1.5	2/10	0/5
35.7	7.6	5/10	0/5

Ozone-air mixtures, by volume, were prepared by diluting metered amounts of ozone generated by passing a known volume of scrubbed air through a modified room air ozonizer with measured amounts of scrubbed air. The air used in these studies was scrubbed by passage through a fritted glass bubbler containing potassium dichromate in concentrated sulfuric acid followed by a column of soda lime-silica gel and a column of Drierite. No impurities were detected in the scrubbed air by this procedure.

Hydrogen peroxide air mixtures were prepared by diluting metered amounts of hydrogen peroxide vapor obtained by passing measured volumes of water-pumped nitrogen into approximately 12 ml. of 90% H_2O_2 with known amounts of scrubbed air.

Hydrogen peroxide-ozone mixtures were obtained by diluting metered amounts of each component with a known amount of scrubbed air.

In order to obtain satisfactory agreement for analytic values for the inlet and outlet vapor-air mixtures, it was found necessary to have about 20 liters of air for dilution or one air change a minute in the exposure chamber.

Ozone determinations were made routinely by a modified alkaline potassium iodide method developed in this laboratory. H_2O_2 was analyzed by titanium sulfate reagent which is specific for this constituent. Since H_2O_2 also reacted with the alkaline potassium iodide reagent, it was first necessary to absorb it in a chromic oxide-potassium permanganate mixture before ozone analyses could be made, using two sampling impingers in series.

During the course of these studies, a considerable difference was again found between the inlet and chamber concentrations of the various vapor-air mixtures. This difference had been noted and was found to be more pronounced with H_2O_2 when animals were exposed. Without animals, there was approximately a 30% decrease for O_3 and a 40% decrease for H_2O_2 when the chamber concentrations were compared with the inlet concentrations. With animals, the decrease was 40-50% for O_3 and about 80% for H_2O_2 respectively. Studies at Army Chemical Center have likewise emphasized the discrepancy between analytical chamber concentrations for H_2O_2 as compared to calculated concentrations. (Rats were found to be exposed to about 1/10 of the expected H_2O_2 concentration) Absorption, adsorption, decomposition, or a combination of these or other factors undoubtedly explain the discrepancy between the inlet and chamber concentrations. This discrepancy was more pronounced with H_2O_2 than with O_3 and was dependent upon the number and species of animals exposed.

Additional inhalation studies to be investigated are to involve aerosol oil mists with O_3 , SO_2 with ozone and acrolein with O_3 as well as other possible air contaminants.

DR. HEIMANN: The next speaker will be Dr. Matzen, who will discuss the physiologic effects and mechanism of action of ozone, work that he has been engaged in for the last year and a half.

DR. RICHARD N. MATZEN: Our ultimate goal in this study is to determine the response of the lung and the cardiovascular system to ozone in acute high level exposures and during prolonged low level exposures. We hope eventually to determine by what mechanism ozone produces pulmonary edema, and the manner in which tolerance is produced.

Up until a short time ago I had been working with mice to study two observations made earlier in this laboratory. First, it had been observed that vitamin C, given just prior to an ozone exposure, reduced the mortality resulting from such exposure. Secondly, it was clear that animals that received preliminary low doses of ozone could survive subsequent lethal challenges that would kill controls.

Vitamin C.

First let us consider Vitamin C. May I have the first slide please.

Slide No. 1

Effect of Vitamin C on Ozone Toxicity in Mice

Av. O ₃ Conc. ppm by Vol.	Vit. C. Dose mg	Mortality		Av. Body Wt. Gm.
		4 hr.	72 hr.	
7.5	10	0/10	0/10	22
	0	3/10	3/10	23
6.0	20	2/100	63/100	--
	0	20/100	84/100	--

This slide shows two runs from the 10 which were done on this particular experiment, and are fairly representative. I think you can readily see Vitamin C is effective in decreasing the mortality to ozone exposure. Another experiment on 200 mice shows a mortality of 5/100 in the treated group and 40/100 in the control group at 5.2 ppm O₃ x 4 hrs.

We next determined the normal pulmonary water content for mice of this species and found it to be 78.4% by weight. This measurement does not vary with body weight nor with volume of blood in the lungs. It will increase with increments of edema fluid as this fluid is approximately 95% water. It was found that the lungs of mice treated with C were less in water content than the lungs of controls simultaneously exposed, and that this difference coincided with the mortality of the two groups. That is, there seemed to be less edema fluid in the lungs of treated animals.

It was conceivable that Vitamin C could act through the cortical steroids in decreasing the permeability of the pulmonary capillaries, or in decreasing the mortality by increasing stress resistance. This is not the case as hydrocortisone actually increases the mortality of exposed animals and the water content of the lungs of these mice are the same as their controls.

As a result of this study it was concluded that:

1. Vitamin C is capable of decreasing the mortality of ozone exposure.
2. This decrease in mortality seems to be related to a decrease in the edematous response in the lungs.
3. Vitamin C does not act through cortisone in increasing stress response, nor does it seem to act through it to decrease capillary permeability in the lungs.
4. Vitamin C does not act merely as a supportive agent.
5. Vitamin C could act by:
 - a. itself decreasing the capillary permeability in the lung.
 - b. reacting with ozone before it has a chance to contact the lung tissue.
 - c. sustaining the alveolar-capillary cells by supporting them during conditions of hypoxia - perhaps by taking part in aerobic oxidation.

May I have the first of the two colored slides please?

This slide shows the lung of a mouse that was exposed to 8 ppm ozone for 4 hours and then killed. You can see the edematous response. The lungs are swollen, there is rounding of the margins, and the surface is of a deep red mottled appearance.

Slide 3 -- This next slide shows the lungs of a mouse exposed at the same time. He had received a previous exposure to 2 ppm ozone 3 days previously, for four hours. In contrast to the previous slide, these lungs appear grossly normal.

Slide 4 -- The next slide shows the increasing protection that is afforded by increasing the degree of the 4-hour pre-exposure dose.

Slide No. 4

Effect of Varying O₃ Pre-Exposure Doses on Lung Water Content and Mortality in Mice

Ozone Conc. (4 hr. exposures)		Mean % H ₂ O Content Rt. Lung	Mortality Numerator: No. dead Denominator: No. tested
Pre-Exposure ppm	Challenge ppm 4 days later		
4.4	8.8	79.9	0/5
0	8.8	87.2	5/5
2.7	8.8	80.6	0/5
0	8.8	86.6	4/5
1.5	8.0	80.7	0/4
0	8.0	83.0	3/4
0.1 day 1	8.8 (day 9)	87.2	5/5
0.2 day 3			
0.1 day 5			
0	8.8	87.2	5/5

There are several things to be noted here. First observe how the water content of the control and the pre-exposed group shows an increasing difference with increase in the size of the pre-exposure dose. Next observe that 3 doses of .1 ppm do not equal one dose of 4 hours to .3 ppm, and that the first difference in the control and pre-exposed groups is noted at .3 ppm pre-exposure dose. This may reflect the activity of the ozone molecule. A certain threshold concentration evidently must be exceeded before protection is afforded. If one boosts

the concentration so that all the inhaled ozone does not react in the upper tract but reaches the lower respiratory units, protection is afforded. It would be my guess that the effect is then one of the terminal bronchioles and/or alveoli.

Further experiments show that the water content of the lungs does not change after a pre-exposure dose - even up to 4 days, although histologically some edema is present 20 hours after pre-exposure. The edema of ozone by the water content method, has subsided 36 hours after exposure to 5 ppm. Tolerance is present within 24 hours after pre-exposure, reaches full effectiveness at least within three days, and lasts at this level up to 102 days after exposure. Following a high level 4-hour challenge, pre-exposed animals often show little or no edematous response, while controls are pouring out edema fluid. Pre-exposure at 2 ppm for 4 hours also gives protection up to $2\frac{1}{2}$ x the LD50 or 20 ppm. There is an indication that the CNS may be affected by exposure to ozone.

Ozone may have its effect in the lungs by a simple necrotizing action on the parenchyma, through damage to the cell wall, or its enzyme systems. Any theory to explain ozone toxicity must also satisfy tolerance.

Interesting possibilities occur:

1. It is possible that as the result of the first exposure the presenting cell surface is altered or the cells or lining fluid may increase in depth.
2. Ozone may react with a native protein to create a foreign protein and through it, or an accompanying histamine type reaction, cause edema, and an antigenic response.
3. As a result of work done by Aviado, Pontius, and Schmidt we know that there are respiratory chemoreceptors accessible by way of inhalation of aerosols. It is conceivable that the stimulation of such receptors causes edema but at the same time destroys the receptor cell. The lack of response on the second exposure is proportional to the number of receptors destroyed on the first.

At present, I am studying the response of the bronchii, the lungs, the heart, and pulmonary circulation to ozone in dogs using open-chest preparations of the heart lung type. These preparations not only allow one to measure changes directly but further to trace these changes to the responsible organ or system. One can isolate the various factors such as cardiac output, lt. heart resistance, vagal stimulation, etc., singly or in combinations. Let me illustrate this:

We have measured an increase in resistance in the lungs following O_3 exposure under conditions of constant volume delivery to the trachea. This essentially reflects a decrease in air space. This type of measurement may be explained by an increase in the blood in the lesser circulation, a broncho-constriction, or increase in bronchial artery flow. The true cause of the increased resistance in the lungs may be further elucidated by corollary procedures such as controlling the blood flow to the lung and the measurement of blood outflow. If no pooling occurs when exposure is initiated, yet the same resistance is present, it indicates that the lesser circulation is not pooled; and this possibility is eliminated.

This example is indicative of the type of study I am now doing. As yet it is premature to discuss results.

DR. HEIMANN: Thank you, Dr. Matzen. The next paper is by Dr. Dobrogorski who will present some observations on the pathology of ozone toxicity.

DR. OLGA J. DOBROGORSKI: I am going to present some histopathological features found in experimental animals after single and repeated exposures to ozone. Very few data concerning the pathology of ozone toxicity have been published. The reports deal mainly with death rates and gross observations of animals that died as a result of ozone exposure.

In order to get additional information, we exposed two groups of mice to low concentrations of ozone: One group was exposed to one part per million, and the other group to three parts per million, both for four hours. Serial sacrifices were performed of mice chosen at random. In mice that were killed immediately after termination of a four-hour exposure to one part per million of ozone no edema was present. In those sacrificed 20 hours after termination of the exposure, a fair number of alveoli were filled with an edematous precipitate indicating a delayed response. In higher concentrations such as three parts per million for four hours, edema developed during exposure and was detectable grossly and microscopically right after the termination of exposure. May I have the first slide?

(A slide was shown.)

This is a section from a lung of a mouse that had been exposed to three parts per million of ozone for four hours, and was killed immediately after exposure. You can see that there is not very much edema that is detectable histologically, although there is some stringy and granular edematous precipitate in the alveoli. It does not stain readily, probably due to a low concentration of albumen present in the edema fluid.

Also there are some extravasated red cells and the hemorrhages that are found grossly were confirmed by histological examination. The next slide please.

(A slide was shown.)

This lung specimen is from a mouse of the same series, that was killed 20 hours later. The quality of the edema fluid has changed. It has become more homogeneous and takes the eosin stain much more readily, apparently due to an increase of protein. Also, there is an outpouring of cells. Polymorphonuclear leucocytes are present in the alveolar walls and some have migrated into the alveolar spaces and around blood vessels. The polymorphonuclear leucocytes, however, tend to accumulate around the terminal bronchioles and ducts. The next slide.

(A slide was shown.)

This is from the same series of mice. The mouse was killed about 66 hours after termination of exposure. You can see that the epithelial lining of the terminal bronchiole is absent. Around the bronchioles there is cellular reaction forming a "cuff." The initially acute reaction changes in 48 to 66 hours into a predominantly chronic type. The polymorphonuclear leucocytes have disappeared and lymphocytes and some phagocytes constitute the reaction. If the exposure is not repeated, the cellular reaction disappears within two weeks.

Besides the edema and bronchiolar reaction a single exposure of ozone causes sloughing of the tracheal and bronchial epithelium with cellular reaction in the walls. The principal site of injury, however, appears to be the bronchiole.

I mentioned earlier the effect of exposures to one part per million of ozone. The sequence of histological changes due to one part per million was similar to that found in mice exposed to three parts per million; the reaction, however, was definitely milder.

In order to investigate the injury caused by repeated low-concentration exposure over a long period of time, we exposed four animal species, dogs, hamsters, rats and guinea pigs to one part per million of ozone, six hours daily, five days a week. This was carried out over a period of 280 exposure days, that is, for over a year. The mortality rate due to pneumonia appeared to be enhanced by exposure to ozone. The death incidence was spread rather evenly throughout the exposure. Rats and guinea pigs that succumbed during the early exposure showed a marked cellular reaction around the bronchioles and desquamation of the epithelium, similar to that described in mice. After about 25 to 30 exposure days, fibrotic strands began to appear in the bronchiolar walls.

The animals that survived the long-term exposure were sacrificed for histological examination. The next slide.

(A slide was shown.)

That is a bronchiole of an unexposed guinea pig of the same age that served as a control. You can see the rather smooth, thin walls and the surrounding thin alveolar walls. May I have the next one as a contrast?

(A slide was shown.)

This is a bronchiole of a guinea pig that was exposed for 280 exposure days to one part per million of ozone and was sacrificed at the termination of exposure. The epithelial lining is hypertrophied in some places; in others, desquamation takes place. Here the bronchiole is completely denuded and is lined with fibrous tissue. The latter extends into the walls of neighboring alveoli. Also there is cellular reaction, both acute and chronic, in the bronchiolar walls, and the lumina are filled with edematous precipitate, some desquamated epithelial cells, and inflammatory cells. The next slide.

(A slide was shown.)

Here you can see a marked distortion of the terminal airways with the epithelium varying from hyperplasia to desquamation; also there is fibrosis and cellular infiltration. The main features in guinea pigs were bronchiolar distortion, and stenosis in some instances, and a mild to moderate degree of emphysema. Presumably the emphysema was not an again phenomenon, since none was found in the controls. The next slide please.

(A slide was shown)

This is a bronchiole of a rat that was exposed to one part per million of ozone for over a year. Some thickening of bronchiolar and ductal walls is present. There is cellular infiltration and fibrosis that extends into the neighboring alveoli. The latter are lined with cuboidal cells, and macrophages containing granular pigment are present in alveolar spaces.

Hamsters showed very similar findings to those of rats, and emphysema was present in both groups. Since the control rats showed the same amount of emphysema we cannot definitely conclude that ozone caused it.

In dogs we did not find any permanent lung changes; neither was there any change in the intrapulmonary airways. There was only some irritation of the trachea with the cilia being clumped together, and some superficial desquamation of the epithelial lining.

It appears that lung changes caused by one single exposure to low concentrations of ozone clear up. Chronic exposure to one part per million of ozone over a long period of time may cause permanent lung damage, such as fibrosis and distortion of bronchioles and alveolar ducts and emphysema. The lung injury is by no means specific to ozone. Very similar histopathological changes are caused by NO_2 , phosgene and other respiratory tract irritants.

DR. HEIMANN: Dr. Scheel, will you tell us some of the details of your work?

DR. LESTER D. SCHEEL: Yesterday afternoon we discussed somewhat the determination of the injury, and Dr. Dobrogorski has now indicated that certainly low concentrations of air pollutants do injure. I would like to discuss now the area where we are trying to measure not total injury as defined by death, but injury which may be defined as any change from normal function or structure resulting from exposure to any causative agent. We have used ozone in this particular case as a matter of convenience, because when I arrived at the laboratory every corner of the building had an ozonizer and it was the easiest accessible tool.

To produce pulmonary injury in the animals, rabbits and rats were exposed to ozone once a week for one hour. I want to emphasize again that we are studying single acute injury episodes and their chronic effect by repeated exposures on the animal. The presence of pulmonary injury has been estimated by an evaluation technique whereby we do respirometry using a closed circuit respirometer filled with oxygen, to measure the oxygen uptake and the minute ventilation, and also to draw a pattern of the respiratory cycle. With this technique it has been possible to measure the mechanical and physical factors of respiration which are involved in pulmonary function. To determine the chronic effect of multiple acute injuries, the ozone exposures were repeated on the same animals once each week for one hour.

(A slide was shown.)

This slide demonstrates the type of results we have obtained with our small animal respirometer from a rabbit. In the lower tracing we have a tracing for a normal rabbit of the same age as the rabbit tracing shown in the two upper tracings. The middle tracing indicates the rabbit before exposure, after his seventh exposure. In other words, he had had one week of recovery after the previous exposure. You can see he has a fairly normal respiration curve and his ventilation is reasonably

good. He was then exposed to 15 parts per million of ozone for about an hour and a quarter, and in this case you see his tidal volume has decreased in amplitude and we have appearing in the stretch tracing here the indication that obstruction, or a nervous reaction producing apnea is present. I believe someone mentioned yesterday that one of the symptoms in humans on exposure to ozone was pain in the chest. I would say that this may indicate the rabbit has a pain in the chest.

The comparative value here of his ability to take up oxygen is indicated by the slope of the line during the closed phase of the respirometry cycle. The ordinate here happens to be cc's of oxygen per minute. If we look at the pre-exposure value of this particular rabbit, which is representative of a group of eight that we have been studying, we have 78 cc's of oxygen per minute consumption before exposure. After exposure his oxygen consumption falls to 42 cc's. We have in this case reduced his ability to take up oxygen by about 50 percent.

I believe that without any further description we can say that the pulmonary function of this animal is very seriously limited. This limitation lasts for four to six hours after exposure, and were it necessary for this rabbit to do physical work he would, perhaps, have considerable difficulty.

Let us go on to talk about what happens on chronic exposure, and here we have used the normal rabbit of the same age with 115 cc's of oxygen consumption per minute to compare with the pre-exposure value of the repeatedly injured animal which has 78 cc's of oxygen per minute. The injured animal has lost about 30 percent of his oxygen uptake capacity.

In speaking of reserve capacity, let us again compare the additional loss over this 30 percent of another 50 percent of that. Using an ear oximeter we measured the oxygen saturation in the rabbits immediately after exposure when this 50 percent drop in oxygen consumption was present, and we found those rabbits at rest to be in normal limits as far as oxygen saturation is concerned. It is my hope that with these beginning changes and the type of pathologic change that Dr. Dobrogorski discussed, that emphysema will develop in rabbits by repeated exposure and perhaps give us another tool to study, a new lesion that has not been adequately studied.

The normal rabbit does not change his oxygen consumption after maturity when the weight stabilization becomes apparent, and this provides a very nice control. These rabbits, three months before this exposure, had an oxygen consumption of about 100 cc's a minute. With the respirometer in normal animals we can measure oxygen consumption with a plus or minus value of ten percent. We are now going into biochemical studies which we hope will characterize the lesion produced by some of the air pollutants and will also give us certain therapeutic suggestions which may be useful. (Thereupon, at 12:00 noon, the morning session was adjourned.)

AFTERNOON SESSION

(The afternoon session on December 19, 1956, was called to order at 12:50 p.m., Dr. Harry Heimann, presiding.)

DR. HEIMANN: Dr. Mendenhall is the first speaker for the afternoon, and the title of his paper is the Effect of Air Pollutants on Mucopolysaccharides and Proteins of the Lung. Dr. Mendenhall.

DR. RICHARD MENDENHALL: One way to consider the effect of noxious atmospheric contaminants is from the chemist's point of view. Logically considered, the initial insult is a chemical one. Whether or not the immediate consequences of this chemical insult can be interpreted in chemical terms, however, is not clear at present. The chemically mediated damage could operate through simple physical processes.

An animal which has been exposed to an irritating amount of ozone, for example, experiences an increased amount of material of a viscous nature in the tracheal lumen. Ordinarily, a normal, non-exposed healthy animal does not exhibit a sufficient amount of liquid material in the lungs to permit one to take samples for analysis. However, rats that have died as a result of single exposures to ozone have lungs from which a fluid can be actually poured. The question as to the source of this fluid has not been answered. It has a high total carbohydrate concentration and exhibits an electrophoretic pattern which is reminiscent of serum. To equate this material with the secretion of the normal Goblet cell response may require extrapolation to a zero dose.

Attempts are at present being made to secure adequate samples of material which may be washed from the living lung without the previous production of histologically evident damage. Equipment has been constructed to this end. Oxygen consumption studies performed on lung tissue may serve not only to indicate living tissue, but may also serve as an indicator of the possible carcinogenic nature of certain noxious gases. The elastic properties of the lungs under study are measured as an ancillary procedure and may yield some worthwhile information. The first slide, please.

(A slide was shown.)

This is the equipment I just mentioned, and in this chamber is a rat lung. The equipment has been designed so that the ambient pressure about the lung, which is totally immersed

can be regulated from the input and the output end and thus make impossible a mistake of subjecting the lung to either too great or too small a pressure inadvertently. This lung has been compressed at about ten centimeters of water pressure. The washing fluid is Ringer's solution. The next slide.

(A slide was shown.)

This slide shows the same lung which has been allowed to expand under a negative pressure, to approximately half its maximum size. Let me point out here the level of liquid in the chamber and the level of liquid in the leveling bulb. The difference in height of those two liquid levels is measuring the intrinsic contractile power of the lung. The last slide, please.

(A slide was shown.)

This is the same lung which has been expanded to its maximum volume, and again the difference in height of the liquid in the chamber and in the leveling bulb is a measure of the intrinsic contractile power of the lung; and you notice that it is considerably greater at full expansion than at half expansion.

The study of mucopolysaccharides is then being undertaken in order to inquire into the source of viscous liquids in the lung which are produced by irritant gases. The nature of chemical changes in the composition of these liquids could point to a mechanism for their formation, and may help us to understand their physiologic effects upon the organism.

DR. HEIMANN: The next speaker will be Mr. Mountain, who will discuss the effects of air pollutants on lung enzymes.

MR. JOHN T. MOUNTAIN: You have already seen that the staff of the Occupational Health Field Headquarters has examined the effects of ozone on the whole animal, on the lungs, and on the tissues of the lungs. Then they pointed the finger at me and said, "You find out what enzymes in the lungs may be affected by ozone."

As part of our routine studies in toxicology we do some work on tissue enzymes. When we started working with the lung, we found that there was not much in the literature on lung enzymes as affected by toxic agents. One report, which came from the University of Chicago described an experiment in which they measured some enzymes in the lung after phosgene exposure

of the intact animal, and the tissues of the animal. So, at least, we had a lead there as to what enzymes might be the ones worth looking at, since they appeared to be most sensitive to irritant agents of a gaseous nature. We went into that because when we tried to apply the procedures we had used on such tissues as kidney and liver, such as measurement of dehydrogenase, we found that the lung for some reason or other did not have as great an activity, and certain modifications as to substrates and carriers would have had to be made. Being a little impatient to get some definite results, and to catch up with the progress made by other members of our Program, we tried to figure out what would give us some kind of an answer in a short time. So, for that reason, we picked on alkaline phosphatase as one of the enzymes to investigate.

Then another thought occurred to us: That perhaps it would be worthwhile to investigate some enzyme which seemed to be peculiarly characteristic of lung tissue. In the outline handed to you, there is given a reference to some work of Reis, who found the enzyme, 5-nucleotidase, which was present in various species and in various tissues and in various amounts. We thought it rather remarkable that in the pigeon the only tissue containing this particular enzyme was lung tissue. That suggested to us that 5-nucleotidase must in some way be characteristic of lung tissue. So we decided to make measurements of that.

From Dr. Svirbely and others of his group who were exposing the animals, we obtained specimens of lung tissue from some of the animals that they had carried through the exposure experiments.

We found, as stated on the outline, that there was a very definite and pronounced drop in enzyme activity, so far as alkaline phosphatase was concerned, after exposure to very high concentrations of ozone. The animals received 17 parts per million of ozone for one hour, high concentration that produces 100 percent mortality. After this exposure, the 5-nucleotidase activity fell to 27 percent.

Then going on down to lower levels of exposure, we found the pattern continued, a drop in the alkaline phosphatase and a drop in the nucleotidase. The alkaline phosphatase fell down to 34 percent of the controls, the 5-nucleotidase down to 62 percent. We are still working with concentrations of ozone that are above those normally found in polluted air.

We had other findings that perhaps complicate the

picture and also perhaps explain and correlate with some of the findings of other members of the group. For example, the activity of both enzymes at 24 hours post-exposure was practically normal. In animals that had been given ozone pre-treatment two weeks before the exposure to the six parts per million of ozone -- they had received one part per million exposure to give them a resistance -- there was evidence that they seemed to have a greater recuperative capacity so far as the enzyme activity of the lung tissue was concerned. Although enzyme activity up to two hours post-exposure was the same as the rest of the group, the rats given the pre-treatment with one part per millions of ozone had alkaline phosphatase levels, 50 percent or more above controls, while the 5-nucleotidase remained at or slightly below control levels.

We are now studying exposures to lower concentrations, that is, to one part per million. We made a preliminary study of that and found that one part per million for six hours did not cause any difference in the enzyme activity of the lungs. However, these enzyme tests were run 48-hours post-exposure. We must test the group immediately following exposure so that we will have to continue our work by measuring effects at low levels, but longer time, exposures and shorter time intervals between the time of exposure and the time of sacrifice of the animals.

I would like to point out some correlations of some of the other work reported here with our findings on the enzyme activities. As you heard Dr. Matzen say, there appears to be resistance to ozone developed by pre-exposing animals to one part per million. That is supported by the findings with these enzymes. The animals that had this one part per million pre-exposure showed much more normal enzyme patterns than the animals that received the six parts per million ozone without the pre-exposure treatment. The fact that 5-nucleotidase is an enzyme possibly involved in nucleic acid metabolism is also of interest, and correlates with the finding of another member of our staff, Dr. Svirbely. He had done some studies on nucleic acid content and that appears to correlate with our findings on the alkaline phosphatase and the 5-nucleotidase.

DR. HEIMANN: The next speaker will be Dr. Fairchild; the subject, the Screening Toxicity of Organic Thiols.

DR. E. J. FAIRCHILD: Toxicologic studies of organic thiols have been initiated as a result of increased usage of these materials in industrial processing of petroleum and recent findings in petroleum technology which indicate that thiols, and/or

their oxidized derivatives, may play a role as atmospheric contaminants in certain types of air pollution.

More work has probably been done with the thiols (mercaptans) in the petroleum industry and a better knowledge of their chemistry obtained than with any other class of sulfur compounds. The reason for this probably stems from the fact that, as a class, the thiols react readily and their reactions are more easily followed than are those of most other sulfur compounds. Furthermore, the total quantity of such compounds in distillates from various high sulfur crude petroleum oils is relatively large. In fact, I believe, if my memory serves me correctly, it has been estimated that if all thiols present in unrefined naphthas in this country alone could be recovered there would be available from 150 to 200 tons per day.

Recent studies by the Petroleum Division of the Bureau of Mines, API Research Project 48, have shown demonstrated approximately 45 different sulfur compounds in a Wesson, Texas crude oil and all of these occur in the 0 to 175° C boiling range. Present evidence would indicate that thiophenes and cyclic sulfides are the predominant sulfur types, but this applies only to the natural oils. Cracking will undoubtedly produce low-boiling thiols and sulfides -- as well as thiophene. Accordingly, our screening toxicity program includes work with certain "type" compounds of the thiols, sulfides, disulfides and thiophenes.

It is surprising, in view of the fact that so much is known of mercaptans concerning their chemical and physical properties, that so little is known in the way of toxicologic information. Apparently, little consideration has been given to the toxicity or physiological effects of the mercaptans because of their odorous character which serves as warning of their presence. However, since these compounds are closely related chemically to hydrogen sulfide they certainly bear watching. Similarly, the importance and multifold actions of sulfur compounds in the animal body strongly indicate the need for investigation. The literature concerning thiol toxicity is rather confusing, inasmuch as certain compounds are classified by one group of investigators as harmless, whereas other investigators classify the same materials as highly toxic.

This report includes toxicity data (work completed to date) on six alkanethiols administered intraperitoneally and orally to rats, and by single 4-hour inhalation exposures to both rats and mice. The results of these tests are presented in the attached table as L D 50 values calculated for 24-hour, 48-hour,

and 14-day post-administering periods. Values represent data from 20 to 25 rats for each compound using 5 per dosage level (50 and 10 in mouse tests). The table should be self-explanatory, therefore I shall not attempt to elaborate on these compounds individually, other than to point out noteworthy aspects of the results obtained.

The thiols tested have the common toxicologic property of being soporific, the degree ranging from mild stupor to moderately deep sleep. General symptomatology of acute thiol poisoning exhibited by rats and/or mice were fairly standard. The toxic signs developed in the order of: restlessness, increased respiration, incoordination, mild convulsions, cyanosis (deep in some cases), muscular weakness, skeletal muscle paralysis in some cases, respiratory depression, and death usually within 24 hours. In cases of maximal sublethal doses the animals usually undergo similar symptoms and recover after a period of lethargy lasting as long as 3 days in some instances. A notable characteristic of certain thiols (especially ethanethiol) is the delayed toxicity shown when administered intraperitoneally and orally in minimal lethal doses. This is readily seen when one compares the 24-hour through 14-day LD 50's in the attached table. This sort of response has not been found for inhalation exposure. The inhalation values given are approximated from work completed to date, but they do show one noteworthy trend. Namely, that the higher numbered carbon thiols are the more toxic by this route of entrance into the body. This, of course, may be associated with the vapor pressures or volatility of the compounds in question since this is a governing factor in the amount of materials taken in by the exposed animals. No marked gross pathologic changes were noted and histopathologic findings are inconsistent with dosages given. There is some evidence that several compounds can cause liver and kidney damage. Death in cases of acute toxicity is attributed to paralysis of respiratory musculature, but this aspect is to be checked by pharmacologic studies.

In magnitude of acute toxicity, the thiols tested thus far do not display marked toxicity for either the rat or mouse. However, by virtue of qualitative response, the thiols may be properly classed (as some investigators have done) with hydrogen sulfide, inasmuch as they apparently have similar action, i.e., paralysis of the central nervous system with death through cessation of respiration.

ACUTE
THIOL TOXICITY

	A n i m a l	COMPOUND						
		Ethane- thiol	Propane- thiol	2-Methyl-1- propane thiol	2-Methyl-2- propane thiol	Butane- thiol	Hexane- thiol	
Intra- peritoneal	24 hr	517	1028	1182	1182	679	600	
LD 50 (mg/kg)1	Rat	48 hr	"	780	"	1029	"	"
		14 day	192	515	917	555	399	396
Order of Toxicity			1	4	6	5	3	2
Oral	24 hr	1034	2362	10,865	5432	2575	2085	
LD50 (mg/kg)1	Rat	48 hr	900	2055	7168	4729	1683	1580
		14day	682	1790	"	"	1500	1254
Order of Toxicity			1	4	6	5	3	2
Inhalation	24 hr	4900				4000	1350	
	48 hr	4600				3600	1000	
	Rat	14day	"			"	"	
LD 50 (p.p.m.) 2								
	24 hr	3000				3700	650	
	Mouse	48 hr	2500			3500	550	
		14day	"			"	350	

- (1) Calculated from 20 to 25 rats, 5 per dosage level
(2) Values have been approximated from data to date

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	Mouse 14day	"				"	350

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DR. HEIMANN: We seem to have caught up with some of our time. Would you like to spend the ten minutes to ask some questions of this last group of speakers? Is there anything that the people who spoke would themselves like to reemphasize?

DR. McEWEN: I believe Dr. Mendenhall said that oxygen consumption studies performed on the lung tissue may serve not only to indicate that the tissue is living but may, according to Warburg, serve as an indicator of the possible carcinogenic nature of certain noxious gases. I was wondering if he could amplify that. Is it Warburg's thesis; that he thinks cancer increases glycolysis?

DR. MENDENHALL: Yes. We want to test lung material first of all after we have worked with it, to be sure we have taken samples of living material. So before and after taking samples of lung tissue by this washing process which I showed you on the blackboard, it would be desirable to study the tissue, not only by histologic examination, but also by studying the oxygen uptake. If one could exhibit maximal respiratory capacity of the tissue, one might also exhibit a decreasing total respiratory capacity by a relatively rapid falling off of respiration when comparing tissue subjected to an irritant with a tissue not subjected to an irritant. Warburg's hypothesis, if I have it correctly, is an appealing one and I think that data of this nature, if it showed a decreased total respiratory power as measured by, say, respiratory capacity over a period of time, could be an indication as to what is happening to the overall enzyme process and its ability to respire, to use oxygen.

I believe some air pollutants are considered to be carcinogenic. I realize, of course, that Warburg points out quite clearly in his thesis that carcinogenic activity depends upon a process of survival-of-the-fittest among the cells which are exposed, and cancer can therefore take quite a period of time to develop.

DR. HEIMANN: Would anybody else like to make some point or ask a question in regard to the last nine papers?

DR. STOKINGER: I might just point out that in regard to this last group of thiol compounds, one of our objectives will be to see what their reaction products are in terms of oxidation with ozone, or an oxidant atmosphere because they are precisely the kind of pharmacologic materials that have profound effects in the body. Oxidation of the sulfur groups provides inhibitors

"of sorts" that are strong bone-marrow depressants, and things of that sort. Therein may lie the value of the study of this group of compounds, not only their toxicity per se.

DR. HEIMANN: Is there any further discussion? If there is no more discussion at this time, we can go on to the next paper. Dr. Sim from the Army Chemical Corps has had some experience with the London episodes and has made some studies in England. In this Country he has continued his studies on sulfur dioxide toxicity at relatively low and moderately high levels. Dr. Sim.

DR. VAN MURRAY SIM: We started out using two methods for exposing human volunteers; one was the use of an oro-nasal mask in which the exposure time was short. The other method was the use of a 100 M³ chamber, for total exposure. The mask exposure periods were 10 minutes because the person was not able to tolerate it for a longer amount of time; the chamber exposures were 30 to 60 minutes in duration.

In using the masks, we cooled the air to -1 to +1° C., in order to see whether or not cold air might add to the problem. We were also interested in the work of Dr. Amdur, who had reported that she had found changes in the respiratory rate, minute volume, and vital capacities of a small group of individuals that were exposed to SO₂. McIlroy, Marshall and Lawther, in two separate studies which we repeated and confirmed, did not find this to be the case. I think, however, that both groups are wrong, because the measurements are made over a "mean time" and the determination is an "average" one; it is not taking into consideration the dynamics of respiratory patterns or respiratory volumes, nor is it recording the retention figures. In extremely irritant atmospheres I am certain that these things do change.

However, after the people have been in breathing SO₂ and SO₃ for a period of time, they become accustomed to it unless the exposure has been altered to either raise the concentration or humidity, or increase the particle size. If one takes the mean of the full period of time, there is not much difference from the normal control, unless the foregoing conditions are altered.

We did all of the routine physiological function tests, and in these tests we found little change in this large group of individuals. We were specifically interested in changes of airway resistance to air flow. We used the Ainsworth interrupter technique and the continuous interrupter technique machine. I am sure that many of you have had difficulty finding that machine and I might explain that fact. It is because Ainsworth does not like to publish his material. To my knowledge, Dr. John Clements, who worked with Ainsworth for a time, has not reported his modification of Ainsworth's machine; so it may be sometime in the future that somebody is going to publish the

specifications of this apparatus. By and large it is a useful machine in that it gives repeatable results. But, if you expose a person to an irritant atmosphere, you will find a marked difference. As you see there (pointing to blackboard) we had quite a number of exposures. We tried SO_2 alone by mask, at levels ranging from 3.6 to 216 mg/M^3 (1.34 to 80 ppm).

The interesting part came when, at concentrations of SO_2 which were in the upper range around 20 parts per million -- we noticed that several things were happening to the men. On entrance into the chamber there was some lacrimation, coughing and sneezing and complaints of tightness in the chest. This did not always occur. At first, we were doing these resistance tests only before and after the exposure; but, we found that we were missing an important part of the reaction, so we started doing them at five-minute intervals all during the experiment, and found that initially, with any of these substances, there was usually an increase of from 35 to 150 percent, above normal in the person's airway resistance; this, then, tapered off over a period of time after they were in the atmosphere, falling gradually to somewhere near their normal figure.

Another interesting finding was that with SO_2 , particularly in the lower concentration, if you elicited any rales by auscultation, you usually heard them in the left third and fourth intercostal spaces anteriorly. There was not a marked degree of wheezing nor were the rales of a musical character. However, with the higher concentrations, -- above 20 ppm -- and in exposures to the wet mist of sulfuric acid, for a period of 35 to 60 minutes, one would find that the rales became more deep-seated, tended to be more posterior-lateral and were more persistent.

When we started with sulfuric acid, we had approximately 10 normal acid and the particle diameter was about 0.9 micron when it was dispersed in the chamber. We were not at all impressed by the irritating quality of this mist. We went on for some time, and were about to give it up, when we decided to change from what was approximately then 61 percent humidity to about 90 to 92 percent humidity. This caused a marked increase in the irritancy of the material. In fact, the men could not be examined by the machine in the first ten minutes after exposure because they coughed so severely. The cough did not quiet down so that they could be put through a proper test until about ten minutes after they entered the chamber. Further, they did not like to stay in there the full 60 minutes. I think that the important thing about the humidity increase is that it leads to larger particle size.

We did some retention studies and found that the dry mist at 9.6 parts per million was about half as irritant as the wet mist at 20.8. The important alteration was a change in mass median particle diameter

from 0.9 micron to 1.5 micron when humidity was increased. What would happen if the particle size goes up further, I do not know; that is one of the things that would be of interest.

Another thing of interest to us was to know what part is played in the exposure by aldehydes and other constituents of diesel engine exhaust. We did not try to assess this by studying all the physiological mechanisms; we tried some of them on a group of persons. The substances that we studied were formaldehyde, acrolein, crotonic aldehyde, butyric aldehyde, iso-butyric aldehyde, acetaldehyde, and propionic aldehyde. Most of them were not very irritating; but, certainly the crotonic aldehyde and acrolein were very irritant. At 0.8 p.p.m. (range 0.805 to 1.22) the duration of time that the men could remain in the atmosphere was not over five minutes, and they were constantly lacrimating and coughing during the exposure. In the case of formaldehyde, there was irritation initially, and then some sort of anesthesia occurs to the sensory mechanisms, unless one is extremely sensitive. They were able to stand 30 minutes at a concentration of 13.8 p.p.m.

We tried the effect of cooling the inhaled gases for a short period of time. We feel that cold may have played a key role in the London smog deaths. Of the deaths that had occurred in London, most did not occur during the first few hours of the fog. Most occurred after a period of time during which the patients were continuously exposed either to cold or at least to lower temperatures than you and I are accustomed to in our homes. I feel that cold must play some part in this mechanism. Certainly these are not the compounds that in themselves are very strong irritants. Certainly, the oxidants, as has been brought out, may play a part in the picture. Free radicals may play a part in it too. The matter is very complex.

DR. STOKINGER: I have a question about the concentrations that can be tolerated. This range here, say 3.6, is that what you started them at and eventually worked up to this? You could not put them in these high levels suddenly.

DR. SIM: This 3.6 indicates the fact that there were a group of people who were in that level of exposure. This also indicates that the group of men were in that atmosphere right from the start. We started at the low level of parts per million, and as we gained experience it was increased.

DR. STOKINGER: On the same individual or new ones?

DR. SIM: No, each exposure group was exposed to a different concentration.

There were 264 exposures to SO_2 , using masks, with a range of exposures from group to group varying from 3.6 to 216 Mg/M_3 (1.34 to 80 p.p.m.)

SO_2 chamber (330 men) Range 2.7 to 62 Mg/M_3 (1.0 to 23.1 p.p.m.)

H_2SO_4 Dry (183 - mask - 4.1 to 39) (1 to 96) p.p.m.
(316 - chamber 2.9 to 39) (0.7 to 96) p.p.m.

H_2SO_4 Wet (40 - chamber 11.5 to 38) (2.8 to 92) p.p.m.

Groups of 8-10 men were used. They were exposed to any one of the range of concentrations which began at the lower figures and gradually increased throughout the 10 months of experimental days. These were acute exposures, in which no man received more than two exposures in any one week.

Now, that brings up a point. Richard Pattle said he was sensitive to SO_2 at the beginning of the experiment. I did not think I was sensitive to anything particularly. I had never experienced difficulty before. So he used to wear a mask when he would go into the chamber. When he received atropine it was of definite therapeutic value. But, after about six months of going in the chamber with these men, I began wheezing. From then on, every time I would come near the chamber, it precipitated a real asthmatic type of breathing which could be heard anywhere in the room, and it would persist for three or four days. Now, this does not mean that I became specifically sensitized to SO_2 or SO_3 . It means that my respiratory tract became sensitive to a lung irritant. I think that any other irritant that I would have been exposed to would have precipitated these symptoms.

Now, there were two other persons outside of the regular volunteer group. One was an asthmatic who worked for Professor Cameron, and the other was Professor Cameron himself. The Professor, after an exposure of less than one minute, was ill for about four days; we had to really work on the asthmatic after he was in the chamber for less than a minute. This was the SO_2 - SO_3 mist. We planned to obtain a group of people from the Salisbury Infirmary who might have fairly small cardiorespiratory reserve. But, after this experience with the asthmatic and the Professor we decided against that. But I will vouch for the fact that I personally can become sensitized after re-exposure.

Lawther and his group were interested in whether or not there was any difference between the population of London and a group that lived in the suburban areas. As you know from the British papers, they took representative samples and could find no differences on single exposures. This makes one think of a number of possibilities as to what happened. I believe you can become sensitized to any of these irritants if you

breath enough of them.

DR. MATZEN: Dr. Sim, could you predict the rapidity or the degree of severity that different people would react to SO_2 by their allergic history, or anything like that?

DR. SIM: There were eight such cases in our series. All of the volunteers were examined, and all of them had a careful history taken in relation to asthma, hay fever, and other allergies. There were only eight who had a personal history of either asthma, or other allergy, and in only two of those did we find it necessary to remove them from the experiment. In other words, these two men could not tolerate it. The rest of them went through all the exposure trials.

I may say this: Those that had asthmatic histories or histories of hay fever tend more frequently to wheeze, and they were the ones who had some of our higher resistance values. I know that this is not a large sample for valid conclusions; it is an impression, however. I would be hesitant to expose known asthmatics to such concentrations as this again. How much part is played by previous daily exposure to low doses followed by the application of a sudden high dose, I cannot tell you. These are just some of the problems which became evident.

DR. FISHER: The next paper will be by Dr. Kotin.

DR. PAUL KOTIN: In discussing the possible role of the atmosphere in carcinogenesis, specifically pulmonary carcinogenesis, I first want to emphatically say the following: I do not believe that any single agent is responsible for the initiation of any neoplastic process. And, secondly, I would like to submit as an axiom that the demonstration of carcinogens in an atmosphere by no means carries the inevitable corollary that it is a biologically active agent. With those two provisos I would like to discuss some of our work in relation to atmospheric carcinogens.

In the beginning, our study was based on the epidemiologic background data as a basis for suspected carcinogens of the atmosphere. We began to pursue the study of the carcinogens and broke them down into the group of polynuclear aromatic hydrocarbons -- members of the dibenzanthracene series. Incidental to our initial extraction studies and identification of these materials, we set up a series of controls and one of these controls included the non-aromatic portion of the atmospheric contaminants. We were surprised when we found the production of skin tumors with a fraction of the atmosphere which contained no aromatic polynuclear contamination, to the limits of our ability to detect, and certainly, to our satisfaction. This observation was made in 1952. We did not believe it. We could not conceive that these materials could have been present for so long a time without their carcinogenicity having

been noted. We repeated the experiment and duplicated the results two years later. I will devote most of our time to discussing the activity of this group of compounds and dismiss the others rather rapidly.

The demonstration of dibenzanthracene derivatives as possible carcinogen was not original with us. Waller and his co-workers had done it at Saint Bartholomew's in London and had also shown it to be present in urban atmosphere. We were concerned not only with the demonstration of the presence of the material, but also with showing whether its presence in the atmosphere was compatible with biological activity (carcinogenicity). The first thing we attempted to study, of course, was the survival of these materials in the atmosphere. We had two reasons for this. In attempting to determine the sources of these materials, we examined the exhaust gases of gasoline and Diesel engines, and we found that there was a specific quantitative ratio between pyrene and benzpyrene that was emitted. But, on sampling the ambient atmosphere, we found a reversal of this ratio. To us this could mean one of two things. Either we were not examining the greatest source of these materials or something was happening to these materials in the air. Subsequent survival studies, unequivocally demonstrated, at least to us, that indeed these are the prime sources. It is what happens in the atmosphere to these materials that provided the answer. This was true whether we were dealing with the dynamic atmosphere of Los Angeles or a reasonably static atmosphere such as we see in smaller non-industrial communities. We find that the ratio of the stability of pyrene to benzpyrene in the air is something like one to four, that is to say, benzpyrene will survive four times as long as pyrene. The significance of this proved to be more than academic, when we learned that in Great Britain on the basis of the work of Campbell and his co-workers, they were attempting to utilize air content of pyrene as the index of the atmospheric pollution by polynuclear substances. We can readily appreciate why they preferred using pyrene as their "indicator" substance, since it is the most easily detected, purified and quantitated. But, nature in its own diabolical way made this also the more labile of the polynuclear materials in the atmosphere. So, indeed, they probably were not getting a true picture of the index of atmospheric pollution with carcinogens since they tested only for pyrene compounds such as coronene or anthanthrene, even more stable than benzpyrene, may well have been the ones that they should have used instead.

Having demonstrated that there is a survival of these materials in the atmosphere for long enough periods to be compatible with their being respired, we then were concerned not only with demonstrating their entry into the respiratory tract but also with their physical states. On the basis of studies done by Dr. Falk of our group, before he came with us and while he was with us, the elutibility of the carcinogen from the soot particle on which it is absorbed is a critical factor. We know also that the smaller the particle size, the more tightly is the adsorptive

bond, and the less readily is it broken. From a biological point of view, non-eluted carcinogen is probably biologically ineffective.

This explained an additional factor to us. Presumably these polynuclears have been in the air since the first cave man lighted his first fire, and skin cancer and scrotal cancer occurrences were evidences that when the bond between the carcinogen and soot could be broken, one could get a cancer. On the basis of some analytical studies, we then determined that perhaps in the last half century, paralleling the increase in pulmonary cancer incidence, we not only have had the traditional soot and carcinogen in the atmosphere, but, perhaps, we have introduced into the atmosphere something that nature spontaneously had placed on the skin but not in the respiratory tract: the elutant. (There is no sebaceous secretion on the respiratory epithelium.) And a subsequent study showed these aliphatic materials filled this bill very nicely.

A second factor we were concerned with was the ability of the eluted carcinogen to diffuse into the cell. This we studied in two ways: By means of actual visualization of diffusion in studying the actual morphology of the cell under the electron microscope; and secondly, by the use of carbon-labelled soot and carbon-labelled pure carcinogens. These isotopic studies are still in progress; but we now know that in the case of a carcinogen -- maybe not the carcinogen -- at least the "label" gets into the cell. Whether or not the labelled carbon atom carries the carcinogen with it as such is something that time will tell. We rather suspect that it does.

Finally, we were concerned with the ultimate metabolic fate of the material once it had been absorbed into the respiratory epithelium. We were also concerned with its absorption, or phagocytosis, by the monocytes and macrophages which are normally present in the lung. Again our isotopic techniques permit us to study this. We have confirmed many previous observations, that the material is lost rapidly from the lung. This does not disturb us, because we know that very soon after painting the skin with a pure carcinogen in skin carcinogenesis studies you cannot demonstrate the presence of the carcinogen within a matter of hours, in some instances.

Feeling that practically nothing was known about the carcinogenicity of the group of aliphatic compounds, we began to study these in great detail. For the sake of simplicity, we can substitute unburned raw gasoline for our aliphatic type of hydrocarbon. In the Los Angeles atmosphere, as has been discussed during the past two days, these aliphatic materials are subjected to photochemical changes in the presence of nitrogen oxides and other oxidants. We were faced with the same problem. First of all, what is their atmospheric survival? What is their chemical nature? All we can say is that studies done by many others

have been duplicated by ourselves. We are satisfied that there are ozonides, peroxides, and hydroperoxides present. We do not positively know that epoxides are present. We do not know whether di-epoxides are positively present. We do know their presence is exceedingly likely. The only reason that I mention these groups of compounds is that the carcinogenicity of the latter has already been demonstrated by others and duplicated by us. The original demonstration was by Walpole and Rose in Manchester, working for the Imperial Chemical Industries where these compounds are manufactured for use in the textile industry.

Little did we realize that we were dealing with a "triple threat" with this aliphatic group of compounds. We demonstrated their carcinogenicity for skin as part of a negative control study. Attempting to see whether they played a role in breaking the adsorption bond between the carcinogen and the soot particle which carried it, we found them to be highly important. The carcinogen-soot entity could do a greater degree of damage more effectively than could the sebum on the skin, which we know can, and does, result in tumor production. To see the fact that these materials are irritants, we have only to refer to the desquamation that is seen in relation to the respiratory mucosa. As I will show in a few moments, they are highly productive of irritant effects in the purely non-specific reactivity of the epithelium in addition to their innate carcinogenicity.

I should emphasize, perhaps, on top of all this, the concept of particle size. Once you have admitted carcinogenicity, particle size is probably the most critical thing that we have to deal with. The ability of a particle to penetrate the upper respiratory tract, then the trachea and the successive arborizations of the tracheo-bronchial tree, is related to the particle size; and more significantly, the percent of retention of particles is related to the particle size. So, if we plot percent retention against particle size, beginning with 5 micra down to perhaps 0.05 micra you will find that you have very little retention until you get to about two to one and one-half micra. Then you have marked retention, getting on down to the submicron sized particles when you have particles suspended in the tidal air until you get to the sub-submicron size where Brownian movement will precipitate the particles. We thought that this was an original observation, only to find that the A.E.C. had worked this out for radioactive particles, and we were delighted to find that we could superimpose our graph on theirs in this regard.

Similarly, Waller in England, where they have a large spectrum of particle sizes in the air, found that the percent of retention had a relation to particle size. His findings were similar to the chart that you see here. We began to study the irritant role of these aliphatic materials for two reasons. First, we know that any particle deposited on the respiratory tract epithelium has a residence time of practically zero. Ciliary movement will start moving it out almost instantaneously;

and, it seemed foolish to study the carcinogenicity of materials which are present for a fleeting moment and whose deposition site is so inconsistent.

(A slide was shown.)

What we did was expose intact animals to polluted air in one series of experiments, and in another series of experiments we isolated tracheo-bronchial segments from the level of the thyroid cartilage down to below the smaller bronchi. I do not know whether we got to the very small bronchioles or not. Very much to our surprise we found initially -- although we should have expected it, and as Dr. Pace this morning noted -- that in response to small exposures, there is an acceleration of growth. Initially, in response to polluted air for one hour in concentrations of from one-half to one and one-half parts per million, the polluted air consisting of oxidized gasoline, we found there was an increased rate of movement of particles, in sizes from one and a half down to one-half micra. The initial response of irritation is presumably an attempt by the organism to get rid of the cause. I will now show the next slide which is a classical example of longer exposure.

(A slide was shown.)

Here we have a control rate of 11 millimeters per minute movement and we see two experimental animals exposed for four hours; in one we had so paralyzed the cilia that at least for the 120 minutes no restoration of movement had occurred; and, in the second experiment, after some 70 minutes, particulate movement began and was at a much lower rate than our control.

This applied to all mammalian species studied, from the mouse up through the rabbit and dog. It applied to the irritants that we used, wholly independent of the chemical nature of the irritant. Quantitatively, of course, they varied. Being a morphologist primarily, and not a physiologist, I attempted to correlate this with morphological changes to see what happened to the epithelium; and, a wonderful sequential dramatic series of things occurred.

(A slide was shown.)

This is a section of normal respiratory epithelium. You see the cilia. The fact that you see several layers of cells is normal. By and large there is no subepithelial inflammation here. The next slide shows the beginning of effect.

(A slide was shown.)

The first thing we see is an increase of mucus secretion. The cilia are still functional at this time and, in effect, we have a damaged but normally functioning, epithelium. The next slide shows a progression of this.

(A slide was shown.)

We begin to see a little more subepithelial edema, much more mucus secretion. The cilia here are functioning but at a much lower order of magnitude than normal. May I have the next slide?

(A slide was shown.)

Here we begin to see even more reactivity: We can see some of the mucus droplets in the lumen. The cilia appear quite normal. They are functioning but at a very low order of magnitude. Here you begin to see the compression of some of the cells by the larger amount of mucus. The next slide takes us one step further, where we get actual lakes and pools of mucus.

(A slide was shown.)

You now see cilia that are completely functionless. The cell nuclei are compressed between the lakes of mucus, and are pushed down.

(A slide was shown.)

The last slide in this sequence shows desquamation such as was described by Dr. Dobrogorski this morning. Here we see the loss of the superficial epithelium. There (pointing) is a pool of mucus that has "spilled" over. I call your attention to the fact the basal cells are essentially undisturbed. This has two implications. First, healing can and does occur with no remaining evidence of prior insult. We cannot find the location where the epithelium was denuded, once we allow them a long enough recovery period to epithelialize.

I think we all agree that carcinogenesis occurs in the progenitor cells and here we have such cells completely denuded of their normal morphological defense barrier. They lost their physiologic defense quite some time ago, and here they have lost their morphologic defense as well. Next slide.

(A slide was shown.)

Here you can see, under low power, complete loss of the superficial epithelium, but an intact basilar layer remains.

(A slide was shown.)

The last slide of this series shows the same section under higher magnification where you can see the marked sub-epithelial inflammatory response. There are here as many subacute as acute inflammatory cells, but the basilar cells are surely completely functional at this time.

I should mention at this point that after we expose the intact animals, in order not to influence the anatomical changes, we sacrificed them in one of four different ways. Either by intraperitoneal anesthesia, by a blunt blow on the skull, by intracardiac introduction of air, or by exsanguination.

We then wanted to see what we could do by localization of particles. May I have the next slide, please?

(A slide was shown.)

You can see here that the upper section of the trachea shows no particular deposition in spite of the fact this animal had breathed soot for some 36 continuous hours. The next slide is from a section made of tissue immediately below the level of the tracheal bifurcation.

(A slide was shown)

You can see the two main bronchi. I should have pointed out on the last slide that we had produced anthracotic nodes, evidence that the soot has gotten in, but the particle size that would normally settle out in the trachea and bronchi are essentially similar to those trapped in the upper respiratory tract. The mystery of the relative scarcity of primary tracheal carcinoma is probably really not a mystery at all. It is not that there is some magical immunity of the tracheal epithelium; it is just that the particles do not settle there. May I have the next slide?

(A slide was shown.)

Here we begin to see the morphologic representation of the physiologic and morphologic studies we saw before. Here is soot accumulated on the epithelium. May I have the next slide?

(A slide was shown.)

This is interesting in that the slide before showed the epithelium in the stage of denudation. Here we have some mucus which has held this material so that we have had re-epithelialization of the bronchus, but the soot is still there. The next slide, please.

(A slide was shown.)

Here is an example showing that mucus can hold the soot in a given area of the tracheo-bronchial tree; since animals cannot cough very well, this tenacious material can remain in one place for a long time. You can see the tremendous subepithelial inflammatory response. This is of significance, we feel, in relation to associated cyclic viral infection. We do not think you can rule out the role of upper respiratory infections or influenzal infections as agents which help facilitate the localization of carcinogens.

After seeing this we went back to studying how the effect could be quantitated. What we did was to expose isolated tracheas to the pollutants in chambers where we maintained constancy of temperature, humidity, and acidity, all of the variables that could be controlled. We found that there was a marked difference between the area of impingement of the irritant and the area of "passive passage", as we chose to call it. If you precipitate particles on an isolated tracheal bronchial strip and, under the dissecting microscope, measure their movement, and then expose them to an irritant, specifically our oxidized gasoline, you find that there is a gradual slowing and then a complete cessation of movement if the experiment continues long enough. If, however, you introduce the material in the localized area you will find that if you deposit particles here, movement will be slowed down because some of it is obviously going to diffuse through the rest of the chamber; but, the movement will be at a very low rate except in the area of primary impingement where there is no movement. You can just see the soot pile up around here very dramatically.

If the concentration has been low enough not to cause ciliary destruction, movement will start across here (pointing) very slowly; but, the movement above and below continues at the slower rate. This we thought was important but not nearly as significant as it proved to be because, on examining human lungs removed at necropsy, we found areas of basal cell hyperplasia at the arborization of the bronchi, at the angulation, at the areas of physiologic constriction, at the sites where impingement occurred, in contrast to where passive passage had occurred.

In addition to utilizing human lungs for this study, we extracted the carbon and soot from lungs of human bodies. This was done simultaneously by Professor Blacklock at St. Bartholomew's in London, and again we were delighted to find a congruency of our data. The soot that was recoverable was free of benzpyrene which we know was in the air in Los Angeles and which was present to a greater degree in the air of London.

The question posed itself; is the benzpyrene perhaps innocuously destroyed in the lumen of the tracheo-bronchial tree, or is it absorbed into the respiratory epithelium with a possible mediation of its carcinogenicity? The next thing we studied was the particle size of the soot,

ash, and inorganic substances as well, that we had recovered. And, lo and behold, the particles fell right into the size range that normally would be retained. We, of course, were reluctant to believe this congruity. We were not sure that the absence of benzpyrene from the soot and ash was not an artifact incidental to the rather heroic treatment you must give lungs to recover soot and ash. You have got to digest the material either as acid or alkaline materials or you can use some tryptic digestion. What we did was run carotenoids along with the lung in addition to the coratenoids that are normally present, and quantitatively recovered the added amounts.

We went one step further and added a known amount of benzpyrene to the lungs, and, give or take a standard deviation, we quantitatively recovered the benzpyrene. The loss was attributable to the process of chromatographic separation. We were therefore satisfied that this material is not only present in the air, it is not only respired, but it also disappears and, more significantly, it probably gets into the cell as well.

We utilized some of these aliphatic materials for tumor induction. I can say, that in addition to getting skin cancers, we were able to show in "A" strain mice, on the basis of a tumor dose titration study, that there was a significant difference between our control animals and our test animals at four weekly intervals, beginning with the 26th week. Then removing groups of mice every four weeks up until the 52nd week, we showed that our pure-air controls were not significantly lower in the amount of tumor-bearing animals. The difference in multiple tumor bearing animals was even greater. Single tumor-rate differences may reflect only the "A" strain inbreeding property. This might be so, but variations in multiple bearing tumor animals are different. The spontaniety of tumor occurrence in "A" strain mice under 52 weeks of life, and particularly the mice we used which were adequately controlled, showed that we could claim responsibility for having induced the multiple tumors. We have just finished a study using C57 black mice which carry, according to the literature, a spontaneous pulmonary tumor incidence of something less than 0.5 percent. (This, incidentally is probably a myth, we believe. They actually carry a spontaneous pulmonary tumor incidence closer to 2 percent.) Again, a significant difference between the normal and control is demonstrated. These were all pulmonary bronchogenic or bronchiolar cancer depending on your authority, not epidermoid cancers. This to us means nothing. If the mouse chooses to react on the level of his bronchiolar, rather than his bronchial epithelium, and if he chooses to react with a glandular tumor rather than squamous type, it is truly the business of the mouse. I would submit that a tumor is a tumor when it meets certain criteria. They are transplantable and they metastasize.

We are now approaching the end of an experiment using rats which have been exposed to this material. We chose rats only to satisfy the purists. Vorwald at Saranac and Finkel and Lisco at the Argonne Laboratory in Chicago managed to get bronchogenic neoplasms, with beryllium in the case of Vorwald, and radioactive cerium in the case of Lisco and Finkel. They are using an allied strain of rats and we trust that we may get some tumors with our rats. If we do not it will in no conceivable way mitigate the concept that these aliphatic materials are not only tumorigenic for the skin, they are equally tumorigenic for the lung, and that their introduction to the atmosphere is wholly compatible with the epidemiology of the increase in lung cancer as has been demonstrated by investigators throughout the world.

DR. DOBROGORSKI: What were the time lapses at which you sacrificed animals and found changes in the mucous membrane of the trachea?

DR. KOTIN: The animals that you saw in the photomicrographs were sacrificed after periods of under two hours of exposure to two to five-day exposures. In the sequence, we have had animals exposed for as long as five days with two-day recovery, three days with a week recovery, a week exposure with a week's recovery.

DR. DOBROGORSKI: How soon does desquamation of the epithelium appear?

DR. KOTIN: You can get desquamation of the epithelium within four hours. You first get ciliary paralysis. Then you begin to see pooling of the mucus and the exact moment at which the pool of mucus desquamates, carrying with it the superficial cells, we have not pinpointed.

DR. DOBROGORSKI: What were the ciliary changes?

DR. KOTIN: The ciliary changes were the classically described ones of matting and conglomeration in mucus pools. There is a very nice slide in which you can just see cilia suspended in a glob of mucus, like mitochondria in a cell; just lying around unattached, but still present.

DR. FISHER: Thank you, Dr. Kotin.

Dr. McEwen and his coworkers of the University of California in San Francisco are studying the effects of air pollutants on the eye. Dr. McEwen, will you give us your report?

DR. WILLIAM K. McEWEN: Unfortunately this project involves a combination of people from several disciplines, and I represent only the biochemist's viewpoint. We also have ophthalmologists, toxicologists,

and pharmacologists. One of our big difficulties involves communications among ourselves. So, I will try and skip those parts that I do not know much about.

Actually we have a three-part project. First of all, we are working with a number of pure substances; later we will produce artificial smog; this is under the supervision of Dr. Hine. He has collaborated with Dr. Middleton, and will attempt to produce the same sort of artificial smog that Dr. Middleton is working with. Dr. Hine is also interested in limits of sensory perception by humans, and is trying to determine the threshold limits of recognition as measured by irritation of the nose, mouth, and eyes, by the production of pulmonary discomfort and central nervous system effects.

Dr. Meyers is studying pulmonary function. I wish he could have been here during these talks because he would have obtained a lot of useful information on lung function. He agrees with Whitridge's work, in which changes due to reflexes in the lung constitute the important part rather than any direct damage to the lung membranes. He is going to continue this pharmacological study under this program.

The eye work is associated with two objectives: One is the clinical and pathological examination; the other is the biochemical. There will be, presumably, acute and chronic exposures, and we will look for immediate, delayed and long-term effects, as were discussed yesterday.

But, of course, we have run into problems. What is smog, first of all? I suppose at first we have to do as most people have done: Take a "standard" irritant, which is not always standard, and then hopefully wait until somebody will come up with other compounds that we can test.

In regard to the effects on the eye, there are substances which are irritants to the eye and there also are presumably the lacrimators that cause tearing by their pain effect. They are called lacrimators in the sense that they affect the eyes more than they would, say, the lungs or other sensory receptors we might have.

The other problem which I would like to talk about is at what level of exposure do we work? Now, there are several levels of concentration at which we can work. There is an absolute threshold in ophthalmology and in the biochemistry of the eye. We are used to thinking in terms of high degrees of sensitivity, at least in the photochemical reaction. The eye can sense somewhere in the order of one or two quanta of light, which is really a pretty small amount, so it does not seem that this kinetic paradox that was mentioned by Chambers will hold. It is quite possible that the threshold for chemical substances may be of the same order if you think of impinging an irritant on a nerve ending. A little higher level may be the sensory level. That is the level, I guess

where people start to complain that their eyes hurt and tears start flowing. As mentioned yesterday, this is the level usually encountered in smog and it may not be associated with any demonstrable pathologic changes or biochemical changes. You might call it the sensory level.

Next, is the level at which you get clinical, pathological, and biochemical changes. This does not mean that you might not have a biochemical change at a lower level of concentration of pollutant than would give sensory effect; but, in general the clinical, pathological, and biochemical level is generally at a slightly higher concentration.

The next level might be that which produces gross changes; and this is what I think most people speak about when they are testing any irritant and drop it into a rabbit's eye, look at it and see very red, irritated eyes, and possibly, loss of epithelium. That is gross change. I mention that level because non-ophthalmologists are apt to work at that level. To illustrate that, most of you would feel that a "normal" rabbit's cornea is in pretty good shape. Actually it is difficult to find a rabbit whose cornea is "normal" and unstainable; that is, whose epithelium is completely covering the cornea. You will find that most rabbits kept in cages under the usual laboratory conditions have corneas of which the epithelium is "spotty." If you stain it *in vivo* with fluorescein and examine it under the slit lamp, you will find that pieces of the epithelium are absent. This gross change has been well studied. One might call it reaction to injury. English workers during World War II especially Pirie, did a study on the effect of war gas on the anterior surface of the eye. At Johns Hopkins, Friedenwald conducted quite a broad study on the effect of war gas irritants on the eye.

So, inasmuch as we could pick whatever level we wanted, we felt that the clinical, pathological, and biochemical level of injury might be most productive. We felt that, perhaps, we would not find any changes at the level of pollutants found in usual smog concentrations; but, at least, we wanted to make sure that smog would not cause any changes that we could pick up at this level of study.

I would like to tell you a little bit about the cornea. There is a stroma that supports most of the cornea. Anteriorly, there is Bowman's membrane, a structureless membrane. Then there are five layers of epithelial cells. Behind the stroma there is Descemet's membrane, another glass-like membrane, and then a layer of endothelial cells. It is this epithelial layer that I referred to as missing in spots in rabbits.

Now, of course, that would give us a little difficult time if we are going to measure changes. But it was found that if the rabbit was exposed to a little acrolein, the next day the cornea was in fine shape. In other words, a little irritation to these epithelial cells caused regeneration. It is interesting to note that one of the ways of treat-

ing trachoma is to irritate the eye and cause regeneration of the epithelial cells. Since the disease trachoma resides mostly superficially, those cells are sloughed off and new growth takes its place.

We can trephine a little section here (pointing) -- say a five millimeter trephine -- take out the disc of epithelium, and in 48 hours it will have grown back. Of course that makes a very nice tissue culture preparation. You can look at it, it is clear and it regenerates rapidly. The material you take out by trephine is epithelial tissue and can be studied enzymatically for its oxygen uptake and other studies.

Incidentally, the cornea has a rather interesting oxygen uptake. It has a 30 percent glucose monophosphate shunt mechanism which is fairly rare. I believe the kidney has a fairly high glucose monophosphate shunt, as does the mammary gland. The stroma underneath the epithelium has mostly anaerobic glycolysis. We have an opportunity to study whatever metabolic pathways we wish.

More important than the oxygen uptake is the energy production. Do these pollutants cause any uncoupling of phosphorylation or any decrease in the energy available to the systems? The measure of energy production might be any synthetic process, such as regeneration of tissue.

The cornea, as is found with a number of tissues, will swell in isotonic solution. In other words, these tissues are hypertonic and they keep from swelling by some metabolic pump-mechanism. If the cornea absorbs water it indicates that its pump mechanism has broken down; it has not enough energy to pump out the water. It will then swell and become opaque. This is an index of breakdown of the energy production.

I want to report one pilot experiment. This is not to be considered as confirmed, because we have not reproduced it; it is suggestive evidence only. If you put a rabbit into an atmosphere of 46 parts per million of acrolein, there is little effect. Maybe one ear will droop. The next day the cornea, as I mentioned, will be clear and well healed. If the eye is anesthetized first, however, there will be a tremendous effect of acrolein. Over the whole cornea the epithelium will disappear. You will get injection of the bulbar conjunctiva. However, if the eye is unanesthetized, there will be practically no effect and you will get healing within 48 to 72 hours. And, of course, the point here is that when the eye is anesthetized, the rabbit holds its eye open, but when it is not anesthetized he closes his eye because of pain. When the lid is closed over the eye, the cornea is not subjected to the acrolein and there is not as large an effect.

DR. FISHER: Thank you, Dr. McEwen. Are there any comments here? We will soon take up the question of irritants as a program activity.
(A short recess was taken)

DR FISHER: The problem of eye irritation has been presented to us as an "unofficial-official" request for action. There is considerable public interest in it and we have had one request from Congress to do something about it. In addition we have had some requests to resolve the differences of medical opinion relating to this air pollution hazard.

Eye irritation has been presented again. You have heard Dr. Chambers mention, very briefly, the eye irritation studies which are in progress now with the Stanford Research Institute group. There is some work going on at U.C.L.A. There is direct work in the Air Pollution Control District Laboratory in conjunction with these other agencies. You have heard Dr. McEwen speak regarding plans for work to be conducted in San Francisco.

There seems to be a general impression that this is a psychological problem. To others, the sense of eye irritation is a specific objective response of the eye to air pollution. Something must be done about it. We have been told that if the eye irritant were removed from the Los Angeles atmosphere, there would not be near as much outcry and pressure for immediate action. You can hear all sorts of things, some based on fact, some rumor, some the usual folklore.

Specifically, we have been asked to develop, or to assist in the development of, an objective test for eye irritation, preferably using an experimental animal. Of the studies in Los Angeles, Dr. Chambers can tell us much more than I. We will be interested in knowing the degree of coordination which exists between those studies and the studies in San Francisco.

In the original protocol, Dr. McEwen, if I may quote from that, please, I understood that Dr. Middleton was participating in the planning of the air pollutant materials you would test.

DR. McEWEN: Yes.

DR. FISHER: So there is that degree of positive coordination between the Los Angeles people and the San Francisco workers.

One question that has been raised is: Shall we sponsor work on an objective test of eye irritation? Shall we have that work conducted east of the Rockies or on the West Coast? There are some resources available to us, including the Occupational Health Field Headquarters Laboratory here. Two or three universities are probably

capable of doing the work if we want to pursue the negotiated contract device. We have an indication of some interest from the Army Chemical Corps; that is, they probably could be talked into studying some part of the problem, possibly. This does not constitute an official request for information. This is philosophy.

The Army Chemical Corps has a background in work with lacrimators, as you well know. At one time when that work was carried on they had one objective and one subjective test. Whether that could be expanded to our present needs, we do not know. We have a number of possibilities of handling this by supplementation either with direct funds or through existing research grants. Those are all on the West Coast now. We have not explored the total optical physiology resources in the area around Washington, or on the Eastern Seaboard.

The question is, what would be your thinking on us charging in and independently trying to develop an objective test for eye irritation? Should we leave that to other workers?

Dr. Chambers, do you have anything to comment here?

DR. CHAMBERS: I do not know that I can add very much to what I said yesterday, Dr. Fisher, beyond this: We have a peculiar situation in Los Angeles, as you have indicated, in which some of us are afraid that the concern with the immediate symptoms of air pollution, loss of visibility, eye irritation and plant damage, are overriding the proper concern for the potential impact of air pollution on general health. Nevertheless, we have to recognize that whenever an individual or a population is suffering from symptoms which they can feel, smell, or hear, palliative measures have to be provided. I am a little afraid we are tackling what could be paralleled by a case of measles, approaching it surgically for excision of the rash. The public sooner or later is going to feel that the spots might be removed but they might be annoyed over having been skinned in the process.

However, right now we have to face the fact that we have either a symptom or an overt effect on the population. The public recognizes it and demands action to alleviate what can be called suffering. It is up to us to do something about it.

Now, this is a case in which normally the role of students of the environment, physicists, engineering chemists, is to supply background material which the medical groups can use in interpretation of responses, physiological, pathological and otherwise. In this particular case I think we have a social pathology which has got to be dealt with in a somewhat different manner. If the engineer is going to effect the proper control of a situation which the public demands should be controlled, he must rely on the physiologists or the psychologists, or, persons in some other such discipline, to provide

an analytical tool which will determine whether the preventive and control measures which are put into practice are, in fact, producing a measurable result. I think, therefore, that it might be considered more from the standpoint of a requirement for "social readjustment", if you want to put it that way, or "psychological readjustment" of the community, than from the standpoint of any real attack on an air pollution problem from the medical or public health sense. This is the extreme point of view.

From the other side of the fence you can regard eye irritation as a direct insult to some part of the anatomy and, therefore, it is a health problem. I am very much concerned about it because every control measure we put into effect -- the extremely expensive controls put on combustion processes in industry and elsewhere, such as the current move to take some very drastic steps in regard to mechanical equipment of the automotive vehicles to prevent the emission of exhausts -- all such things are aimed specifically at removal of eye irritation. They may result in alleviation of some of the other potential problems simultaneously; but, we have no means at present of judging the effectiveness with respect to eye irritation of any of these actions except the rather nebulous panel-type of study which is laden with many psychological pitfalls governed partly by publicity media. Frankly, I would consider a test that could be carried out on a rabbit far more objective than one that could be carried out on a man, for the simple reason that a rabbit cannot read a newspaper.

DR. FISHER: We would have to, of course, come up with an animal test which could be correlated with the human subjective report.

DR. CHAMBERS: It obviously has to be correlated with the human subjective response in some manner before it can be used as an index.

DR. FISHER: Let us say it is a request for a biological index which could be referable to human complaint without the intervention of the human.

DR. CHAMBERS: In effect we are asking for an analytical tool which can be provided only by the group concerned with physiological and morphological responses in the human organism.

DR. HEIMANN: Not all of us have had the opportunity of experiencing this "eye irritation." The term "eye irritation" can mean different things to different people. I wonder if some of you who have had the eye irritation as it occurs in Los Angeles, can tell us specifically what it is like.

DR. FISHER: Paul?

DR. KOTIN: My eyes peculiarly are immune to lacrimation.

DR. DAVID: I have some eye irritation right now; just the type of thing that I have in Los Angeles. It is the same sensation, that I have when I am in a smoke-filled room. There is some sensation of burning along the edge of my lids. There is some mild tearing. I never have too much tearing. Coupled with that, in my case, I am aware of some nasal burning, just as one who smokes too much. At least I have had it from that. I am very much aware of it, and it is accentuated when someone else in the room remarks about eye irritation or someone else with me is aware of it. Then, I suddenly realize that I have had it for a while. I do not know if that is subjective or not.

DR. FISHER: Art, have you been in it?

MR. ARTHUR STERN: I have, and I will agree with the explanation given.

I would like to extend a little bit on some of our needs for what I would rather call a bioassay technique which is analogous to what the folks in water pollution have, a technique which allows you to use some form of animal or vegetable life as an assessor of the environment.

It would be useful if, whatever this technique is, it would give us an assay of levels which are less than irritation. In other words, you said you did not want 29 degrees in your scale. To my mind, to be useful you do want the 29 degrees in your scale. To be of the greatest use, that I think any assay technique of this nature would be to us, we must know what and how much pollution is in the air when the irritation is not evident. The assay technique should tell us that we are at half of the level necessary to produce irritation, for example. If the assay technique were sufficiently critical to be correlated with actual irritation, it should be good enough to be scaled down towards zero. Then it would be of tremendous use to us in telling us how close we are to this irritation.

DR. FISHER: As a future refinement, yes. As with the remainder of the physiology of the optical damage here, we are being asked to provide an objective basis for something being done about the output from automobiles during deceleration and idling, which is stated to contain substances irritating to the eye.

DR. McEWEN: I was thinking that this is sort of a neurophysiological problem and maybe should be examined through the "firings" from the sensory nerves transferred to the oscilloscope.

DR. MUSGRAVE: Has anybody studied the blink frequency as an index of irritation?

DR. McEWEN: That may be useful. I happen to prefer the pick-up of the impulses. If the nerves are telling you that something is irritat-

ing, then it tells it by the frequency of the impulses that it sends back to the brain. So, if we can pick up the number of impulses it sends back to the brain, this will indicate the amount of stimulus that that nerve ending is getting.

MR. MANOS: I would like to see some measure of the magnitude of the problem by asking the private citizens in Los Angeles to report on the eye irritation on a mass scale -- something like 2,000 unpaid observers marking each day on the calendar. Say, you send them a sheet of paper with 30 blanks --

DR. FISHER: May I take the opportunity of my being in the Chair and saying that as a rule that does not appear to me to be an objective test.

DR. CHAMBERS: This has been tried on several bases, including TV and the employment of these concerns which professionally carry out market research and census investigations of various sorts. The one thing that makes it appear to be very definitely not an objective test is the fact that it is strongly affected by whether or not there is an announcement in the paper the previous night that there is going to be smog.

DR. FISHER: Dr. Hodge?

DR. HODGE: Might one study the changes in the capillaries of the eye?

DR. FISHER: We are looking for suggestions. Should we be involved in development of an objective test; should we supplement that by saying we are going to do it and then to say where and how we are going to do it?

DR. HEIMANN: Is it possible to have an objective test at all?

DR. FISHER: We are looking for a test, as yet undefined, in an animal which is observed at the same time that 99.44 percent of the people say, "My eyes burn."

DR. CHAMBERS: I think the reception of light is primarily neurological. That has been studied very effectively, as was indicated, down to the last photoquantum, by tapping the nerve and following the physiological processes in the nerve. I do not believe that is a necessary barrier.

DR. MENDE: Perhaps it is true that the effect is partly neurological. However, there must be chemicals involved because the neurological response involved can vary according to the chemical stimulation. For example, the eye could be itching but not smarting, or responding some other way to different chemical stimuli. So, I think that there are still chemical factors that could be explored as to what type of chemicals would cause this type of neurological symptom.

DR. SCHOETTLIN: Dr. Chambers mentioned yesterday visual performance or performance based on visual needs. A task using humans, utilizing an increment of their production norm, might be an index that would indicate something.

DR. CHAMBERS: That type of approach has been explored in a preliminary way by one of the groups at U.C.L.A. with some promising results.

MR. STERN: Is there any indication that there is a correlation between irritation and seeing? Is that what you are saying?

DR. SCHOETTLIN: One of my current problems is to find out if job performance is decreased because of air pollution. Offhand, I think that it is.

MR. STERN: That is not the same thing as correlation with seeing. This is an overall job performance.

DR. SCHOETTLIN: Many jobs are on a visual level where the hands do what the eyes tell them to.

DR. MUSGRAVE: An upper respiratory infection can also affect your eyes.

DR. CHAMBERS: But an upper respiratory infection is usually not as transient as these episodes of smog. It might be possible to differentiate.

DR. KRACKOW: Well, of course our problem in the Army Chemical Corps relates to different types of agents. We have a human engineering group and our psychologists there have been studying the effects of some of our agents on light perception under dim light conditions, and this has been put on a highly quantitative basis. Your problem of eye irritation looks like a problem that may well be attacked in the same way. But to start with, it looks like it is going to be a problem for the comparative physiologists working with the psychologists, ophthalmologists and toxicologists. It is going to require a high degree of correlation of different disciplines brought into play, and, I think a start can be made by a good psychologist or physiologist interested in the eye.

Of course, the comparative physiologist, I mentioned, can bring into play some lower forms of animals. Maybe some of the ciliated animals will provide the basis for an initial index on their behavior. Then we could tie this into what we see happening to man. I think both the animal and the human has to be brought into the picture here.

DR. FISHER: Paul, do you have an idea?

DR. KOTIN: Just that, as Les mentioned, if we could be sure that the same thing that inhibits ciliary activity was responsible for the eye irritation, we would have a third decimal place quantitative test waiting to be picked up. We do not, however, know that the relation exists.

DR. CHAMBERS: We need an analytical tool which would validate that correlation once and for all. This tool is the missing gap. How do we bridge it?

DR. KOTIN: I do not know that anyone has tried to.

Hear Hear
DR. CHAMBERS: That I am sure is the case; nobody has really made an effort.

DR. FISHER: Which brings us back to our proposal that a physiologist work out the problem in a laboratory for a while. Dr. Hodge, would you like to get into this?

DR. HODGE: I might offer one comment. It has been my impression that the ophthalmologists in their research are characterized by an extraordinary expertness in physiology. Possibly, if the word were passed around that you were interested in the development of an objective eye irritation test, this might appeal to the physiologists among the ophthalmologists and you might then find suggestions coming in. Or, perhaps there would be people who would give you observations that they made some time ago that never were followed up and that might be the basis for study now. In other words, I am voting for you to undertake the search for an objective test.

DR. McEWEN: May I take that back to the Proctor Foundation for Research in Ophthalmology?

DR. FISHER: I wish you would. We will leave it at that for the time at least.

DR. CHAMBERS: I will pass the word.

DR. FISHER: Then we ourselves will not plan to start anything directly in the East at this time until we have a lot more information. The skills that were discussed as possibly contributing to such a study, are in existence between Los Angeles and San Francisco.

You each picked up a photostated copy of a memorandum directed to Mr. MacKenzie on October 18, signed "Arthur C. Stern", together with a copy about the fractionation of air pollution sampling as done at the Sanitary Engineering Center.

(Reading) "We are currently fractionating our organic benzene extracts from airborne particulate samples obtained from the network into seven major fractions which are separately reported and are then turned over to the chemical research section for more detailed analysis. It would be most helpful to us if we had some indication as to which fractions contained the substances of greatest hygienic interest, and which were relatively innocuous, so that we could concentrate our analytical work on the more important fractions. For this purpose, it would seem desirable to use some of this material for animal screening experiments.

"For the purpose we have in mind, we believe that pooled samples, regardless of the city of origin, should be used in any initial screening, and that they should be screened for both acute and chronic toxicity. If some of the fractions show toxic properties, we could then obtain samples specific to different cities, as well as finer fractions or specific substances isolated from the fractions.

"In addition to the above, we have on hand approximately 200 grams of benzene extracts from approximately 40 cities which have not been fractionated into the above seven fractions, but which could be fractionated if needed. This amount of benzene extract is continuously growing as the result of our day to day activities. If we were requested to fractionate this material, we would most likely have to hire an additional aide for the purpose, the cost of which would have to be worked out jointly with the Community Air Pollution Medical Program.

"Since we do not extract the entire filter, but retain a portion of the filter against future analytical needs, we have available several thousand partial filters, properly identified, which could be extracted and which would yield approximately 200 additional grams of benzene extract. Here, again, if we were requested to work up this material, an additional aide would be necessary and his cost would have to be worked out with the Community Air Pollution Medical Program.

"Finally, we have facilities for obtaining special large volume samples, such as come from 'Big Bertha' which is now in St. Louis, so that we can obtain a very large amount of extract, if needed.

"I would like to put this matter on the agenda of the next SEC-OHFF Liaison Meeting at OHFF on October 30, at which time Dr. Fisher is to discuss his program. Since this agenda is being set up by Dr. Fisher, this should be discussed with him to find out if he wishes to take some of the time of the Liaison Meeting for this purpose."

This was to be placed in the Liaison Committee Meeting. We decided at the Meeting to present this for discussion here.

I shall go ahead to some considerations. We have considered the possibility of using this material, which has been stored for some time, some of it for carcinogenicity studies. We have not arrived at a final decision yet. The Cancer Institute is interested in this as far as they know the material and the subject matter now, and the possibility for the future. The specimens will probably go to Dr. Heuper.

We have a suggestion for reconversion of this material back into its original volume, its adsorption onto carbon particles, and introduction into test animals as an aerosol.

We have the following suggestions:

"Use of small laboratory animals, specifically the mouse or, where sufficient material is available, the rat.

"That acute toxicity studies not be made; instead, that toxicity tests be made for a chronic, repeated dosage basis as long as sample material is available. This will permit the development and more ready identification of tissue change.

"That the daily dosage be based on the amount of material in each fraction, calculated back to that amount which is contained in 20 cubic meters of air and/or some multiple thereof, samples permitting. 20 cubic meters of air is taken as representative of the average 24-hour intake by adults.

"Interpretation of results: If no response is obtained from such a dosage schedule as revealed by histologic examination of critical organs of the animal, and provided sufficient period of administration has been made, at least 30 days, one might reasonably conclude that the particular fraction in question may not possess sufficient toxicity to be of immediate interest.

"It is to be recognized that this procedure is one of expediency based on limited amounts of materials and is not the procedure of choice if larger amounts of material or more highly fractionated materials are available."

And then there is the suggestion for testing carcinogenicity or co-carcinogenicity.

Mr. Stern, would you like to say something or would you like to see what is presented from the floor?

MR. STERN: I would like to explain why this memorandum was prepared. We have a large air sampling network covering most of the United

States, and we have to determine from time to time what direction it should take. We need to know whether to continue to sample in certain cities and what analytical work-up should be given the samples, because it is expensive to keep a network going and to do all the possible analytical work. We need to re-evaluate procedures periodically to determine whether we are properly spending public money in what we are doing. We would like to have medical people tell us whether network samples have any significance for the health of the populace and what chemical work-up we should give the samples to allow such significance to become apparent.

DR. GAST: I would like to ask one question in regard to this flow sheet. You mentioned the benzene extract. Where is it on the sheet? All I see is the benzene eluate.

MR. STERN: I would like to introduce Mr. Elbert Tabor who is in charge of the network, and Dr. James L. Lodge, who is in charge of our chemical research. They are available for any questions you may have concerning the network.

MR. ELBERT TABOR: That is shown in the very top rectangle, Dr. Gast, the "crude sample." That is what we call the benzene extract.

DR. FISHER: Dr. Mende, do you have any reaction to this?

DR. MENDE: No.

DR. GAST: I have another question. Has there been any correlation yet between the fractionation and what had been found on the mass spectrograph?

MR. TABOR: The report on the mass spectrographic work just came in, I believe the latter part of last week, and we really have not had an opportunity to review it.

DR. GAST: Well, these fractions have been analyzed then?

MR. TABOR: The mass spectrograph work was done on a few of these fractions. I believe they were reported on the aromatic fractions, shown down at the bottom of the sheet.

DR. GAST: That has been done then?

MR. TABOR: I believe that was for two cities.

DR. LODGE: And each sample represented a three-months period.

DR. FISHER: Mr. Tabor, would you care to comment on the possible contents, the number of compounds in each of these categories listed on the memorandum?

MR. TABOR: There is a stock answer to that.

DR. FISHER: It is the same one I gave yesterday about M.A.C.'s.

MR. TABOR: Outside of that, I have no comment. I do not think anyone could speculate as to the number of compounds present.

DR. FISHER: Dr. Lodge?

DR. LODGE: I will take as one specific example, the strong acid group. We are working on this specifically, as an example. All acids containing between one and ten carbon atoms are present, and there is a large group with greater than ten carbon atoms. We have not yet fractionated some of these test samples. The mass spectrometry data will also give us some idea about the aromatics, which really hold up. They do not tend to break down badly in the mass spectrometer. Still, all mass numbers between about 150 and 350 were present.

In summary, the answer still is, anything under these respective categories in the whole group could be found, and probably will be. The important thing, I think, would be the relative amounts.

DR. FISHER: Mr. Krackow, would you care to comment at this stage?

MR. KRACKOW: Not right now.

DR. FISHER: Dr. Chambers, did you want to say something?

DR. CHAMBERS: In the first place, I would like to point out that when this program was initiated, -- and I had some part in its initiation -- its primary purpose was quite apart from the one which is being suggested now as a by-product of it. The purpose in setting up the network was essentially to develop some general interest at the local and state level in problems of air pollution.

The program started approximately three and a half years ago, considerably prior to the enactment of Federal air pollution legislation, and I think it has served the purpose of getting some general backing which has led, at least, to conferences such as this one.

Having operated on a "hurry-up" basis, primarily to elicit some form of activity and interest among a large group of influential people, apparently we have at the same time accumulated a considerable amount of material. It seems a pity that this material should be wasted since it has been collected so laboriously and expensively. Nevertheless, there are certain things that have to be borne in mind. In the first place, we are fully aware that materials of this sort, collected in mass and

manipulated by rather gross procedures in the laboratory, in their final results do not necessarily represent the compounds which existed in the atmosphere. Therefore, from the standpoint of acute toxicity studies, I am rather reluctant to see any effort put on these materials. We know that, even a number of hours after collection, the chemical properties of the substances change progressively. The business of massing them in order to get sufficient amounts for possible studies for carcinogens, and the like, raises a serious question.

I have no doubt, on the basis of the demonstrated presence of benzpyrenes and the like, that the materials will prove in all cases to be somewhat carcinogenic. This, I think, can be taken for granted. One value of this type of collection, it seems to me, lies in the different toxicologic properties between samples that were collected in different communities.

To pool all of this into one mass would simply tell you that some place in the United States we have this kind of a response with this sort of material after this kind of manipulation. So, I am a little reluctant, on the basis of the toxicity or carcinogenicity of the more stable compounds, to see the possible value of the comparison from one area to the other eliminated by pooling in order to get enough sample to carry out a carcinogenic study.

My impression is, from what we had previously supplied to Dr. Hueper for specific study with relation to one or two localities that had special interest, that it would require amounts of the order of five grams in order to carry out a satisfactory study of the type he was conducting. As a matter of fact, in the one area in which the infrared pattern very early revealed the presence of a rather unusual situation, (actually during inversions in New Orleans), caffeine was found to be present in the atmosphere to such an extent that it crystallized out on the sides of the test tubes. It constituted a very high percentage of the total benzene extract. Dr. Hueper was interested in this because some German work had indicated the carcinogenic properties of effluents from coffee roasting. We collected a hundred pounds of effluent from the stacks of coffee roasting plants in New Orleans, and Dr. Hueper's group now has that under study in exposure chambers, with animals continually exposed to a reconstituted aerosol of it for 18 months or longer.

VOICE FROM THE FLOOR: Might it be the chicory they use in their coffee there?

DR. CHAMBERS: No. We checked that. The chicory effluent is quite different.

DR. FISHER: Paul, would you care to speak here.

DR. KOTIN: There are some unpublished data, the details of which are available, showing that the study of such material certainly is valid in epidemiology. There is a population group exposed to this effluent. I do not think that anyone can be wholly certain whether this is really as outlandish as it sounds; but, it would seem to me that it may be a useful approach in view of the fact that we must perforce deal with the stable compounds in this group.

In Great Britain, in both the studies at Leeds and at St. Bartholomew's in London, as well as in our own studies where atmospheric pollutants have been used in a carcinogenicity experiment, with few exceptions the tumor yield has been greater than one would anticipate on the basis of what we now know about tumor dose studies. I do not know how significant or how valid tumor dose studies are; nevertheless we glibly say, "this constitutes a 75 percent tumor dose of 3, 4-benzpyrene, or 100 percent 90-day dose of 1, 2, 5, 6 dibenzanthracene", and the like. On that basis we felt that the test would fill the gap, would explain the additional tumor yield; but, it still has not.

I would certainly be interested in obtaining the weak acids, the strong acids, the bases and the water soluble materials for the purposes of reconstituting them, not in terms of what their presence was in the urban atmosphere from which they were collected -- because, again, nature in its own diabolical way usually makes this too toxic to use -- but, a representative relationship as a basis for reconstruction would give a lot of valuable information. It would not require the full 36 months of the life of a mouse to evaluate them. Whether right or wrong, there is at least some highly impressive work from the University of Pittsburgh, that most of the so-called co-carcinogens are, in effect, very weak carcinogens, per se. This is the subject of some controversy. But quite quickly, in 6-9 months I think, one could determine whether these things had any cancer-promoting effect and if the weak and strong acids had a cancer-promoting effect. I know that it must sound like heresy to say that an organic acid may be a carcinogen. We really do not know whether they are or not. I think we could, in a reasonably short time, eliminate them as adjuvants. If a substance does not act as a co-carcinogen, you have to stretch pretty far to ascribe innate carcinogenicity to it. As long as this material is available for the asking, I am putting in by bid for a portion of it.

DR. FAIRCHILD: Dr. Kotin, would it not be required to use combinations of 1 and 2 and 3 of all of those?

DR. KOTIN: Absolutely.

DR. FAIRCHILD: You would have to run it all the way through.

DR. KOTIN: In carcinogenicity studies the initial expense is not the difficult one. It is the housing of a group of laboratory parasites -- which is what animals are -- while you are waiting for them to develop tumors. That is expensive. You would not have to wait too long, I believe. I would be satisfied if one would be able to demonstrate a promoting effect within a given period, arbitrarily saying six to nine months. In some instances three months could be enough, if you perform croton oil, or other parallel types of carcinogenic control experiments. The necessity for long-term carcinogenic experiments with these materials could be, to some degree, obviated. The negative data would mean something immediately. The positive data would then require laborious experimentation. But just to eliminate them on the negative basis would be, from my point of view, a tremendous thing.

DR. FISHER: Dr. Stokinger, would you like to comment?

DR. STOKINGER: I agree with Dr. Kotin.

DR. CHAMBERS: Do you see any way in which this could be done in a manner which would result in comparative information from one metropolitan area to another, so that with the development of subsequent morbidity and mortality patterns there could be correlation? Or, are you thinking of this in terms of being pooled?

DR. KOTIN: No, definitely not pooled.

To begin with, for co-carcinogenic studies you need very small amounts. And, secondly, I think the availability of large numbers of positive and negative controls would give validity to the experimental data on the basis of the first run. There is enough traditional information available with this type of study so that unlike most other air pollution studies, you can by-pass the exploratory phase.

MR. STERN: I would like to note here that this material does not exist at the moment completely pooled. We can pool it and combine it.

DR. KOTIN: These substances may very well explain the complete, and at the moment unexplainable, difference of the epidemiological findings of the U.S. Public Health Service, in which Atlanta and New Orleans, presumably geographically and culturally comparable areas, have contrasting lung cancer incidences. We know it is not due to difference in smoking habits. We know that because the people in New Orleans do not smoke differently from the people in Atlanta. At least one has a valid right to assume that.

DR. FISHER: Dr. Gordon, we continue to abuse the privilege of your presence here. Would you care to enter the discussion here?

DR. GORDON: From an epidemiological standpoint I would be impressed by the desirability, particularly in light of the London experience where there is so much variation in air pollution from time to time and place to place, to put emphasis on examinations in a smaller area first and then spread to the more universal approach.

DR. LODGE: The particular interest that my group has in this is the following: We are distinctly limited in our time, money, and personnel. We, ourselves, have no objection to any necessary pooling to get necessary sizes, for the following reason: If it can be shown that the interest lies in a certain fraction, we have a certain set amount of manpower that we put into preparing a detailed breakdown. There is no particular mystery about how to fractionate these samples. There is some cut and dried work which is necessary to go into it. Nobody is going to argue about the carcinogenicity, for example, of the aromatic fraction. We know already that it has benzpyrene and dibenzanthracene, and so on, in it. We have pretty good evidence on that at this time. If we can be furnished with information on the probable toxicity of the other fractions, we can give priority to those of greatest interest. We can have at least a first breakdown of the 20 predominant species in any one fraction inside of a relatively few months and give you that list for your examination.

I would not deny that you may already have the information on the particular chemical species involved. It may be known, but we cannot attack all of these fractions at once.

DR. GAST: Is there any information on the nitrogen content of any of these fractions?

DR. LODGE: It is relatively slight except for the amines themselves. And, I would guess that there is some in the water soluble portion. Primarily it will be in the basic group there. I would not state flatly that the oxygenated neutral group is free of amines.

DR. FISHER: The question has been raised before, about the possibility of finding sensitizing agents. It was not in the original list of considerations.

DR. HEIMANN: From what you said, Mr. Stern, I understood that some pooling of the samples has already been done.

MR. STERN: By city only. I mean that material collected on different dates in the same city has been put together.

DR. HEIMANN: So, for communities there is no pooling. Is that correct?

MR. STERN: I think that is correct.

DR. HEIMANN: We have been talking about the possibility of testing this material for carcinogenicity. But, we have not discussed the possibility of testing this material for other toxic effects. That seems to me to be as important. What I had in mind was that if we still have the material by community, we should consider testing it for toxicity. If it turns out that there is, for example, more of a kidney damaging agent in a sample from one area than there is for the material from another area, one could then investigate to see if there was more human kidney disease in that community.

DR. KOTIN: This, I think, could not actually be done because I would very much question whether even individual groups of compounds are likely to result in the demonstration of any, heretofore, undiscovered compound. So, in effect, we have here a group of stable compounds, any of which probably have toxicologic significance, that have already been studied by virtue of their just being pulled off the shelf or by virtue of their having had some toxic implications. What you are dealing with here is a group of stable compounds, and, I think, quite necessarily, toxicologic studies would have to be done on the materials which are relatively unstable.

DR. CHAMBERS: Worse than that, I think many of the stable compounds you would be dealing with in studying toxicity, would be compounds which in fact have never existed in the air.

DR. STOKINGER: You are talking about working with these large numbers of fractions from different cities. The problem that you must solve is whether you are going to put your time, effort, and money into personnel to do the toxicological work or to do the fractionation; would it be better to make the fractionation and then make the decision as to whether or not it was interesting enough to test toxicologically?

DR. HEIMANN: If it turns out that the data that our statisticians are working up for us now, show some peculiarity in kinds of morbidity or of deaths, should we for those communities do toxicological studies of some of these samples?

MR. STERN: I believe Dr. Chambers stated that many of the substances we get on analysis never existed in the air. I do not believe that this entire group of materials was manufactured in our test tubes and was completely non-existent in the atmosphere. I find it very hard to believe that this whole complex which we have in our samples was all our own making and that we have "brewed up" the stuff out of some very elementary material which we are sampling out of the atmosphere. The primary materials in the air have already well reacted while in the atmosphere and most of the reaction occurred before we actually obtained

the sample. I do not doubt some reaction also occurred afterwards; but, I have yet to have it proven, and I would rather disbelieve that the bulk of the reactions take place after we have gotten the sample. I think most of it has occurred in the atmosphere, and, therefore, this represents the stuff which we are breathing. Undoubtedly, there were reactions which took place in the atmosphere from the time of the emission of effluents from some source, and other things which happened before we breathed it; but, until shown otherwise, I am convinced that the stuff which we have in our samples represents pretty much the stuff which we are getting into our lungs.

DR. CHAMBERS: There are two lines of evidence which have demonstrated rather conclusively that new compounds are formed, and I would not maintain for a moment that the bulk of the material is altered. I think the bulk of it is probably chemically inert. But, in the first instance, a series of chemical tests have been performed, starting immediately after collection of certain samples of this type, which have clearly demonstrated that even such gross manifestations as the acidity or alkalinity changed progressively with time of storage after collection.

In the second place, for these very samples that we are talking about, the chemists who were preparing the materials for infrared examination on several occasions identified entirely new compounds by infrared technique, -- materials appearing as a result of the manipulations that were done in the preparation of the sample. So, I think it is unreasonable to expect that the highly reactive peroxides, and other types of compounds which we are talking about as present in the air, can maintain their identity when combined with such a conglomerate mixture as this represents. I think it is unreasonable to expect that, and I do not know of any evidence that proves they do maintain their identity.

MR. KRACKOW: Generally the toxicologist likes to know that the materials to which he exposes animals truly represent the operational atmosphere. I am afraid that if a laboratory took these samples and went through the conventional type of toxicological study, results would not necessarily be meaningful and applicable to the air pollution problem. I think it would be unwise in this instance to attempt to transfer a chemical problem to the toxicologist in the hope that the toxicologist can get a good answer with a minimum of effort from the chemist.

DR. LODGE: I get the impression here that an initial, rather simple, proposal on our part is being inflated into something cosmic and then is being vetoed because it is cosmic.

DR. FISHER: There has been no veto. If we had a decision from the Supreme Court, I am reasonably sure we would have a split decision.

DR. LODGE: Well, let us say "torn down" because it is cosmic. Am I to infer from the discussion that has gone on here, that those people exist here who are prepared to make or to inform us right now whether that class consisting of all of the strong organic acids in the atmosphere is or is not of toxicological significance? We are simply asking for a relatively elementary simple screening job to enable us to establish working priorities on the basis of which we will be delighted to come back with specific analytical methods for those substances which we find in these classes for which you give us priorities. Currently, as a matter of practice, we picked the strong acids because they are relatively easy to work on, and the aromatics because we knew that here was a "trouble spot." What about the other classes of materials? This is our question: Which should we attack first?

DR. FISHER: Dr. Hodge, do you desire the testimony of other witnesses before you speak? I think I have about exhausted the supply.

DR. HAROLD C. HODGE: Dr. Fisher, in the presence of so many experts, to bring in someone who is on the periphery is really asking for it.

DR. FISHER: Should I preface this by saying, you have been asked for comments.

DR. HODGE: Yes, I have been asked for comments. I am reminded of the young man who got up under a similar occasion and said, "It is probably impossible to exaggerate the importance of this subject, but I will try."

The acute toxicity test for a chemical compound is, to the toxicologist, very much like a melting point is to an organic chemist. It is one of the ways he earmarks a compound. Generally speaking, it does not have any extended value. Generally speaking LD50's do not ordinarily help directly in the solution of problems of public health or industrial personnel protection. The LD50 is not a very important "working number." To know anything about acute toxicity, a reasonable amount of experimental work must be done and the amounts of materials that are listed in this memorandum simply are not sufficient. I shall stand corrected by Mr. Krackow, or any other toxicologist here, who may wish to debate the point. But our laboratory would not tackle screening toxicity testing with such amounts, except under extraordinary circumstances.

// Hear
Hear

Furthermore, even if we had, say, 200 grams of each one of these fractions, I will stick my neck out and predict that the LD50s will lie between 50 and 500 milligrams per kg body weight. Now, this really is sticking my neck out. We all know of extraordinary things, because there are some most unexpected variations in toxicity that crop up; I

should dig myself a good foxhole before I leave this statement. As an instance of an unexpected toxic component, one may take the pyrolysis of Teflon, polymerized tetrafluoroethylene. You may heat that mixture up to 500 °C, and higher, and get all kinds of compounds, compounds of carbon and fluorine. Most of them have acute toxic doses of maybe a gram per kg (or more). Anyway almost all have low toxicities except perfluoroisobutylene which is about ten times as toxic as phosgene. How in the world could anybody predict that?

So, it is possible that if we made LD50 measurements on these fractions, we might find one to be exceptionally poisonous. If so, maybe it would be worth the effort. However, as Dr. Chambers has pointed out, and several others have substantiated, we do not really know that what is now in those test tubes was the molecular form existing in the atmosphere or, if it was in the atmosphere, whether it was responsible for some danger to health.

Let us take a look at chronic toxicity. There is more reliability on chronic toxicity data than on acute; but, there is not enough material in any of these fractions to test the chronic toxicity. Dr. Chambers talked about 100 pounds of effluent from coffee roasting. You can do chronic studies with 100 pounds but not with 4 grams. If toxicity tests were to be made, I would suggest that we go to the trouble of collecting large samples and use them for chronic exposures, studying the animals carefully to see if there is any specific kidney injury, heart injury, collagen injury, etc. My feeling, as I went over this outline, was that, as Mr. Krackow pointed out, this problem certainly is one the toxicologists should hand back to the chemists.

The identification of the components in the samples seems to be very important. Possibly, a chemist with a good imagination can go back from what is now in the test tube to suggest that certain chemical groups were present in the original material in the air; at least to account for the kinds of materials that are found in the samples. It is possible that the substances present in the largest amounts are not the ones that cause the health hazards. It may be a trace material that is really the dangerous one.

Furthermore, even when we expose experimental animals in chronic tests to these substances, we might not find the answer. Dr. Stokinger and his colleagues in our laboratory tried unsuccessfully for a number of years to develop, in the experimental animal, the chronic pulmonary lesion of beryllium poisoning found in the human. We never did see diffuse pulmonary granulomatosis in our experimental animals. So, there is no guarantee that these expensive and difficult chronic exposures would reproduce the human disease. The possibility that there are highly active materials here, certainly is an attractive one; I wonder if there might be ways of trapping them. In the last issue of the Journal of Bio-

logical Chemistry, from Elgin's laboratory in Oslo, there is reported that the anti-irradiation sulfhydryl compounds (e.g., cysteamine) may act by a combination of the sulphur of the cysteamine with a protein in such a way as to protect an essential group, the one that the radiation ordinarily destroys. Perhaps something like that can be going on in smog exposures. Is there some way of trapping the active component in the atmosphere?

So, Dr. Fisher, my comment is that acute toxicity testing would have doubtful usefulness. There is insufficient material. Chronic toxicity testing might be useful but much larger quantities would be required than are now available, except for carcinogenesis studies. The chemical analyses of these fractions, with the purpose of trying to work back to what might have been in the air, is, to my mind, a very important procedure.

DR. FISHER: Thank you, sir. We move now to the oxidant problem. Dr. Hodge, you were not here yesterday. At that time I said that we were coming to no administrative decision at this meeting. We are only collecting opinions from the audience.

The oxidant problem, the ozone studies about which you heard yesterday and this morning, have come up for consideration in the total program, especially their relationship to the Air Pollution Medical Program responsibility. We have mixed feelings here. There is a considerable amount of ozone toxicology work going on. I do not know to what extent the work elsewhere reflected the finding in your laboratory of the combined action of ozone plus other oxidants.

We see ozone work being carried on in direct relationship to industrial exposures, accidental exposures, Air Force interest, and finally the community exposure. I wonder if anybody has any opinion he would like to give us, or to make any comment, on the degree in which we should intensify, decrease or maintain at the present level our ozone investigations. Dr. Chambers is already on record as saying that it is still a problem which they must work with in California. They will continue to give attention to the problem there. I am reasonably sure the first three groups I mentioned will continue to work on ozone.

Does anyone have any idea whether we should go on with the oxidant and ozone levels, looking for health effects? For example, we might still suggest to Drs. Pace, Gast, and Deichmann that they might be concentrating more of their studies on oxidant type pollutants. Does anyone have any quick answer on that or any ready comment?

DR. STOKINGER: I would like to say that in the use of ozone as it appears to be over-extended, it is merely used as an example of a

typical respiratory tract irritant. We are going to work out the mechanism of lung irritation. Whatever may be important to the development of this idea of the mechanism of tolerance is important not alone for ozone, but also from other inhaled gases; this may also be a primary mechanism of response for them too. After the outlines and the basic findings are made with ozone, we will progress to other important gaseous constituents and to other materials in the vapor phase.

DR. FISHER: We have discussed that fully and I am in agreement. The only question was: Is there any direction in the ozone-oxidant studies that we should be taking that any of you think of; any suggestions outside of those that are currently under way?

DR. SCHEEL: Dr. Fisher, I would just like to reiterate Dr. Lodge's idea that oxidation and reduction atmosphere is our one way of chemical classification. I know this did not appeal to everybody yesterday and it probably will not today.

DR. FISHER: On the contrary. After the meeting I heard some people endorsing his idea.

DR. SCHEEL: In the absence of anything better to use as a classification basis, we at least have this one idea on which we can begin. And, there are present in the literature a series of oxidation-reduction types of reaction which could be used to classify oxidation-reduction potentials of atmospheres, when properly applied.

1. This involves the usual risk in any kind of research that the bulk of the data which you collect may not mean anything at the end. But, in the same sense, and with an equal chance, you have the possibility that it will mean something. I am in hopes that it will mean something. Most of our biological mechanisms, both plant and animal, happen to be reducing systems, and this reducing system is brought to light in the practical sense in that the plant damage around London is not quite the same as the plant damage around Los Angeles. Therefore, the oxidation-reduction type of classification may bear some practical usefulness when properly applied. I, for one, would like to see that system given a fair chance to see what it will yield.

DR. McEWEN: I thought that the body reaction ranged all the way from oxygen down to DPN 1, one of our lower coenzymes; whereas in the plant it is just the opposite, but it follows the same general pattern, produces oxygen and goes down to about DPN 2.

DR. CHAMBERS: I was always under the impression that the colorimetric, and some other methods which have been used in characterizing the atmosphere in more specific terms, are in fact oxygen reduction indicators. They can be interpreted that way, at least.

*Something here
unread + study*

DR. GAST: I am a little disappointed after Dr. Tebbens' beautiful example of the source of the air load of organic materials to talk simply of oxidation and reduction of air pollutants.

If you include the particulates, I think you have the potential for reactive systems and compounds that do not necessarily allow classification solely on the basis of oxidation-reduction potential. I think that Dr. Hodge's excellent example of the non-toxicity of a lot of the fluoro-compounds, is another example that does not fit. Midgely picked on Freon as non-toxic, when everybody was sure that the fluoro-compounds were going to be so very toxic. However, if you pyrolyze Freon, you get considerable irritation, but, it is all due to hydrogen fluoride, as far as I know. But what I am getting at is this: These reactive materials may lie anywhere along the scale of the oxidation-reduction potential. They are probably going to be specific for specific physiological or biochemical reactions.

DR. SCHEEL: I agree with Dr. Gast that the classification scheme is not an answer in itself. But what we are dealing with in air pollution is not the air pollution of California, although it has been highly advertised, but with the air pollution of Nashville, Tennessee, Birmingham, Alabama, Pittsburgh, Pennsylvania, Chicago, Illinois, and Detroit, Michigan, and other cities. The classification is based only on the chemical reactivity of the total contamination load.

The study of surface activities and their effect upon chemical reactions, I am quite familiar with, and I agree with Dr. Gast that this brings in entirely new areas of reactivity about which we know very little. This particular area is one in which detailed work will have to be done. But, if we find that eye irritation is greater in California simply because the air has a higher oxidant capacity than in London, where the oxidant capacity is at the other end of the scale, so to speak, then it seems to me we have one correlation with irritation. I speak only of oxidation as being more irritating than SO_2 , simply based upon the LD50 dose of ozone as compared with the LD50 dose of SO_2 , for an example. The reactivity of ozone is much greater, to be sure, than the reactivity of SO_2 . These are only expressions of orders of magnitude. The oxidation-reduction classification must be a basis for an order of magnitude comparison of the total mixture, of which I am no more enlightened today than I was a week ago.

DR. FISHER: This has been difficult. I think we have benefited in a number of ways and to a considerable degree by this meeting and the discussions. We thank you very, very much for your presence here.

(Thereupon, the December 19th Session was adjourned at 4:25 o'clock p.m.)

The December 20 session was called to order at 9:25 a.m., Mr. Arthur C. Stern presiding.

MR. STERN: This final session will come to order. We will call for the reports of the four Chairmen of the several non-medical panels.

We will start with the Agricultural Panel, and I will ask Dr. Middleton, of the University of California at Riverside, Chairman of that panel, to report for it.

DR. JOHN T. MIDDLETON: It is a real pleasure to express gratitude to my colleagues for their help in the preparation of this report.

Since it is a bit awkward to read such a report, I will comment on it and pick out some salient features for your consideration. (The text of the report appears in Appendix "A".)

I call your attention to the first paragraph of the report which states that air pollution causes significant damage to agriculture in this Country. Mr. MacKenzie alluded to this yesterday. We require some figures on dollar loss as a result of injury to plants and farm animals from air pollution. We point out that plants are useful indicators for detecting the presence of air pollutants and can be used to delineate their geographic distribution. We call particular attention to this for possible use by medical groups. The production environment and genetic composition of bio-assay materials largely determines the effects of the toxicants on them, and, because of these factors, it is virtually impossible at the present time to set up maximum allowable concentrations. In fact, it is utterly ridiculous to establish such levels at this time. The toxicants present in the atmosphere may be measured with some ease in certain instances; but, their ultimate effect upon biological material is determined not alone by the concentration of the toxicant but also by the other environmental factors in which the plants and animals are grown.

It is necessary at any meeting like this to segregate certain items that are more important than others; and, we have taken this liberty. We have pointed out that fluorides constitute perhaps the most important group of pollutants in the Country and are responsible for significant economic damage to farm animals and crops. In emphasizing fluorides, we do not mean that sulphur dioxide and diverse organic compounds are less important, but rather that research activity in organic and inorganic fluorides is considerably more inadequate compared to the size of the problem.

Perhaps the next most important airborne toxicants are the oxidants such as ozone and nitrogen oxides, and oxidized organic materials with which they may act as a complex. This group of materials is somehow involved in the smog complex which occurs in some urban areas and is responsible for various deleterious effects on plants and animals.

Ethylene and perhaps other low molecular weight unsaturated hydrocarbons are believed to produce adverse effects on a variety of agricultural crops, including flowers and other ornamentals. This injury occurs at concentrations in the parts per billion range. Ethylene does not adversely affect animals in this range of concentration.

There are miscellaneous organic materials, as represented by aromatic complexes and phenolic compounds, which may have adverse effects on plants and require some study.

We relegate a rather low order of priority to the oxides of sulphur simply because there has been very excellent work in this theater of operations for a long time. There have been very significant advances made in understanding the biological effects of sulphur dioxide in the past 75 years.

Other air-borne contaminants, including the heavy metals such as mercury, lead, arsenic, selenium, as well as a group of miscellaneous materials, such as hydrogen sulfide, mercaptans, ammonia and carbon monoxide, are considered as possible air pollutants causing damage to plants and animals.

Please note that most of the fluoride research needs deal with the profound effects of the contaminant on the physiological processes, the function of the organs, in both plants and animals. These systems should be studied from both the plant and animal point of view. There are certain studies of cell permeability and respiratory systems that may be related to both plants and animals; but most other research areas require individual application to plants, in one case, and animals in the other.

We emphasize again the importance of environmental and genetic composition upon the bio-response to fluoride uptake. The analytical methods are fairly adequate for the analysis of fluoride in plant tissue; but, there is need for some assistance in the development of systems to sample for fluoride in the atmosphere, as well as in animal tissue. The detection of fluoride in animal tissue is rather complex, and serious attention should be given to this problem.

There is real need for the study of sulphuric acid aerosols. We omit the use of the term " SO_3 ", in this case, and point out that the areas that need attention include the effect of sulfurous and sulphuric acid aerosols and sulphur dioxide on animals, as well as the mechanism of their toxicity, singly, together, and in combination with other toxicants. The specific effects of sulphur dioxide are fairly well known but we do not have much information on sulfuric acid aerosols and their interrelations with sulphur dioxide.

The oxidants, we believe, are the principal items responsible for the "smog" type of damage. We have tried to minimize the use of the word "smog" because it has been defined as referring to smoke and fog, neither of which condition is important so far as the "smog" problem is recognized in most metropolitan areas of this Country.

Although some of the oxidants have been identified as ozone and nitrogen oxides, the agents responsible for most of the injuries to vegetation remain unknown, though they are believed to be oxidized organics arising from photochemical reactions in the atmosphere. From this you will perceive that the plant-damaging agent in smog is unidentified; that it is not nitrogen dioxide and is not ozone.

There is real need to develop analytical methods for their identification and determination, as well as to study the mode of action of the toxicants, their effect on metabolism and growth in plants, and possible effects on animals. We bring up the possible effects on animals because there may be an increased difficulty with respiration in farm animals, particularly poultry, in areas associated with the metropolitan areas of California. This should be investigated.

Organic materials comprise a group of contaminants which have adverse effects on plants and possibly on animals. We point out that ethylene, and perhaps other light-weight unsaturated hydrocarbons, may be intimately associated with significant economic damage to orchids, floricultural and ornamental plants as well as a variety of other agricultural crops. We believe that ethylene is damaging to some plants at concentrations in the parts per billion range, while no effect is recognized in animals at this concentration. A critical need exists for analytical techniques suitable for the detection and monitoring of ethylene and related materials. This is one point that needs emphasis.

Although mercury has a low vapor pressure, it has been known for some time to be an air pollutant, responsible for plant injury in enclosed spaces. The recent introduction of mercurial paints for use in greenhouses has renewed interest in this problem and has emphasized the need for re-evaluation of this toxicant.

Similarly, other elements, including heavy metals, are known to produce adverse biological effects, and we list a few. It is interesting for those concerned with public health, that plants have the capacity to absorb mercury from the atmosphere at concentrations well below those normally detected by instrumental methods. Mercury is accumulated in plant material at a fair rate and at a level which is usually not detectable by most methods for atmospheric contamination by mercury. Here, then, is a biological system that is useful for detection of mercury at low levels.

Hydrogen sulfide, mercaptans, ammonia, nitric acid and carbon monoxide, while toxic to plants and animals, are usually not important except when there is a gross accidental discharge to the atmosphere.

We are concerned with, but did not include, radioactive materials as air pollutants in this discussion.

The U. S. Department of Agriculture must augment funds available to existing research facilities and management services so that they may actively study, from basic and applied points of view, the factors affecting the occurrence and result of air pollution upon agriculture. The Department of Agriculture with the Public Health Service should support liaison with private, industrial and public research organizations. The basic problem the agriculture group is concerned with is an environmental problem, and there already exists in these two agencies, and many others, groups that are concerned with the environment. Rather than establish special air pollution agencies, we feel very strongly that this work can be materially emphasized by using existing groups, to have them become interested and apply themselves to the problem at hand. It is important, indeed, that sufficient interest and, if necessary, financial support be given to the development of good liaison between private research institutes, industrial research organizations and the public health organization. If confidence can be established between these several groups, the millions of dollars that have already been expended in air pollution studies may make available to all concerned the accumulation of research data by these groups. This confidence, I am frank to state, does not presently exist. It must be developed. The Public Health Service and the Department of Agriculture can supply the leadership and direction required and surely should apply themselves to the problem with vigor.

We omit the "price tag" for the simple reason that the amount of money necessary depends upon where the work is undertaken. If certain groups are already concerned with the environment, they have a capital investment, and it would be much cheaper to pursue the program there than it would be to set it up anew somewhere else. Knowing that you did not have any money to disburse now, we made our work easier and stopped early. Thank you for the opportunity to present our views.

MR. STERN: Is there anything anybody wants to add or are there questions to be directed to the Committee through its Chairman?

DR. HEIMANN: What plants accumulate mercury from the air?

DR. MIDDLETON: There is a large variety of plants that accumulate mercury. A very useful one is the rose. A variety of weeds also accumulate mercury. Dr. Zimmerman, do you have some others you can think of offhand?

DR. ZIMMERMAN: There is a long list of plants which are sensitive to mercury vapor and can be used to detect the presence of mercury in air. The one most commonly referred to is the rose, since it was the first that was found to show symptoms when the air of a greenhouse became polluted with mercury vapor. The symptoms showed first on flowers. The well-known Briarcliff pink petal appeared faded, and eventually turned brown. When the pollution was great enough, the small buds were inhibited, and black patches appeared on the flower stems. Leaves usually drooped and developed abnormal tissue along the veins. All of the tissues absorbed the mercury from the air where bichloride of mercury had been applied to soil to kill earthworms. In another case mercury compounds were added to paint used for the inside of the greenhouse. When in contact with organic matter metallic mercury was formed. Since metallic mercury has an appreciable vapor pressure, there was enough mercury vapor liberated to damage the plants in the greenhouse.

DR. STOKINGER: Has consideration been given to the idea of the adaptation of plants to certain types of smog? I think you have a different problem here than with mammalian hosts because of the more rapid turnover in generation time. Has any thought been given to that?

DR. MIDDLETON: We have considered the adaptation of plant material to urban pollutants, particularly organic materials. A farmer has as much difficulty moving away from a polluted area as another business man. He usually has a sizeable capital investment; but, there are a number of materials that can be planted in polluted areas that are resistant to pollutants. Also, the farmer has the opportunity of regulating some portions of the environment, such as soil moisture, fertility, and other factors that predispose plants to damage. These alterations reduce the deleterious effects, but, the only real solution is the abatement of the toxicant in the atmosphere. Is that what you had in mind?

DR. STOKINGER: I was thinking more or less of the adaptation of seedless grapefruit and modification of adapted plants that would handle significant, but no high, concentrations of certain types of smog.

DR. MIDDLETON: There are certain plants that are already developed that can be used and that are resistant to oxidants. Other plant varieties are being selected that are resistant to smog and we hope to greatly expound this research program.

MR. STERN: Does any member of the Agricultural Panel object to the publication of the report? (No objection)

MR. STERN: The next to be heard from is the Chemical, Aerosol, and Instrumentation Panel. The Chairman who is going to report is Dr. Lewis Rogers of the Air Pollution Foundation, Los Angeles. (The text of the report appears in Appendix "A".)

DR. ROGERS: We established somewhat different "ground rules" in our Panel than the Agricultural Panel followed. We suggested specific projects. We also indicated approximate size of the projects by use of the letters "S", "M", and "L", meaning "short", "medium", or "long" range. By "short" we implied a project that would require one or two "man-years"; and, "medium" as something requiring two to five "man-years"; and, "long" requiring five to ten "man-years", or more.

We did not get to establish priorities. We were urged in our panel, particularly by some of the people from the Taft Center, to try to place priorities on the projects. We did not get to this.

The preamble of our report states that we have tried to consider projects which are already going on and have tried not to duplicate them. We emphasize that there should be a great deal of attention given to sampling and analysis. In both the aerosol and gaseous field it became apparent that some better system needed to be developed by which methods of analysis could be standardized.

This might become, it seems to me, a program that the Taft Center could foster. This would be a project to develop standard samples and standard methods. Moreover, inter-laboratory comparisons and statistical evaluation of these methods should also be provided. I am particularly aware of the problem that we have in the case of ozone sampling and analysis. At the moment there is really no reliable chemical analytical determination for ozone in the parts per hundred million range.

Will you refer to the list of the aerosol and gas phase projects. The total number of projects is fairly large. There are 16 in the aerosol area and 19 in the area of gas phase studies and interactions. This is a total of 35 projects, and if you add up the "L's", "M's" and "S's" and apply a man-year factor together with a monetary factor, this becomes a very large "package".

There were a number of people present on our panel who had particular competence in the aerosol field, and they suggested specific projects which are listed. The numbers in the paragraphs below that list correspond to those of the project titles.

1. "Mechanism of smoke and fume formation" including methods of modification relates to aerosol generation or modification as it issues from stacks.

2. "Formation of aerosols during photolysis of polluted atmospheres." This has reference specifically to the West Coast problem. We are still uncertain as to how aerosols are formed, but there is a reduction in visibility during smog periods. This has not been studied very much by photochemists.

3. "Application of the particle size spectrometer to nucleation studies." This has reference to Dr. Alexander Goetz' new device that he calls a "particle size spectrometer." This project would explore the possibility of modifying the particulates entering the spectrometer by various manipulations.

4. "Relationship of the composition of aerosols to that of the 'feed' material." This has a specific example in the relationship of particulate sulfate to sulphur dioxide in the atmosphere. Another example is that of the relationship of particulate nitrate to the gaseous and liquid nitrogen compounds in the air.

5. "Changes accompanying the aging of particulate pollutants." This differs from the first project which has to do with the specific formation or modification of aerosols in stacks; this project would involve the modification of atmospheric aerosols.

6. "Development and evaluation of devices for the controlled production of aerosols." There were several interesting suggestions made by the panel, including arc techniques and furnace methods.

7. "Physical properties of aerosol systems." This involves determinations of surface area as well as other properties such as light scattering.

8. "Effect of aerosol shape on light scattering." It may be possible to get some idea of the aerosol shape by looking at a single (aerosol) particle simultaneously with two photocells, oriented at 90 degrees to each other. The intensity of the light may be related to the shape of the aerosol.

9. "Dynamics of movement of particles in large volumes." This begins to overlap into the meteorology panel's projects. I should add here that we had a one-hour session with the meteorologists at which time we discussed some projects of mutual interest. I felt that we needed more of this cross-checking with the agricultural, engineering, and medical panels, but that there was no mechanism or time for doing this. We need "feedback" more between the various disciplines.

10. "Development and evaluation of tracer techniques." There were several people who felt that existing tracer techniques were too slow and expensive. Some suggestions for improved procedures were made as shown on the project list.

11. "Behavior of particles while approaching collecting surfaces." Just how does a particle behave as it approaches very near to some kind of a surface, for example in an electrostatic precipitator or a filter medium of some kind?

12, 13, 14. The titles of projects 12 through 14 were considered to be sufficiently self explanatory as to require no further elaboration.

15. "Application to aerosol analysis of special techniques." There are a great many physical methods that have not been fully exploited in the air pollution field as yet, and what is needed is another major advance with some of them. Let me give an example. I think that the work at the Franklin Institute of Philadelphia with the long path infrared cell is a major contribution in the air pollution field. If some of the other physical methods listed here could be similarly expanded and applied, a definite step forward would be made.

16. "Inter-laboratory comparison and statistical evaluation of analytical methods." One way this could be done is by sponsorship by the Taft Center. We felt that there should be something of the type that is being done by such organizations as the American Society of Testing Materials, the Association of Official Agricultural Chemists, and the American Public Health Association. The problem which those organizations face is that the work done is nearly always voluntary and the comparisons of method is often done by an interested person in his spare time. I think we need a mechanism that is more definite and better organized.

Turning now to the gaseous reaction projects noted in our report, we find that these are all "medium" and "long" range. This is because we knew that there were several organizations working on photochemical projects relating to air pollution, and duplication would be ill-advised.

1. "Photolysis of organic compounds in oxygen." Since we are concerned with reactions in air, photochemical studies should be in the presence of oxygen.

2. "Rates and mechanism of elementary reactions in the photolysis of nitrogen dioxide with hydrocarbons in oxygen." At present we have to speculate about many elementary reactions and their rates. This project would aid our understanding of atmospheric chemistry.

3. "Photochemical reactions of effluents from known pollution sources such as auto exhaust and incinerators." It is my feeling that more studies such as this would be profitable.

4. "Thermal reactions between ozone and unsaturated organic compounds." The first three projects mentioned above have to do with photochemical studies and the next three have to do with thermal studies.

5. "Rates and mechanism of reactions occurring during incomplete combustion." This is a long-range project that would be concerned with, among others, the detailed composition of automobile exhaust. This is a considerable analytical problem.

6. "Nitrogen fixation in combustion equipment." Many kinds of combustion in air will produce nitrogen oxides, and we need to begin looking at the mechanism and factors affecting their formation and destruction.

7. "Collection of specific contaminants by means such as ion exchange resins, molecular sieves, and so forth." We need methods for both collection as well as concentration of pollutants.

8. "Adsorption techniques." This includes the use of materials other than ion exchange resins.

9. "Removal of specific substances such as water and carbon dioxide." This is badly needed in the sampling of air. Those working in the gas chromatography and infrared fields are especially concerned with this problem.

10. "Development and evaluation of methods and equipment for low-temperature condensation of pollutants." This has to do with improvements in "freeze-out" traps.

11. "Development of specific economical, analytical methods." "Economical" is the key word, here. Specific examples are given on the project list.

12, 13, 14, 15. The titles of projects 12 through 15 were read but not elaborated upon.)

We need techniques which will have sensitiveness of an order of magnitude better than those we now have, and one specific suggestion was made that the long path infrared technique could be expanded to ten times its present sensitivity. If this is done, perhaps one could detect odorants, phytotoxics, and the lacrimators.

16, 17. The final group of projects relates to the interaction between gases and aerosols and includes 16, "Photochemistry of organic and inorganic aerosols", and, 17, "Heterogeneous reactions on surfaces." If you have a solid or liquid particle and it has active material adsorbed on its surface, what are the reactions which may take place on this surface?

18, 19. (The titles of projects 18 and 19 were read but not elaborated upon.)

The gentleman who did much more than I on the project summary was Dr. James Lodge of the Taft Engineering Center. The others who worked on this project summary were Mr. William E. Scott of the Franklin Institute, Dr. Jack Calvert of Ohio State, and Mr. Elmer Weaver of the Bureau of Standards.

MR. STERN: We have a minute or two that we can allow for comments or questions. If there are none we will move ahead. Is there anybody here who would have any objection to the publication of the report of the Chemical, Aerosol and Instrumentation Panel? (No objection)

Then, we will move ahead to the report of the Engineering Panel, which will be presented by Dr. Leslie A. Silverman of Harvard University. (The text of the report appears in Appendix "A").

DR. LESLIE A. SILVERMAN: We thought the best thing to do was to state an objective first and then to stress equally the problem of control by engineering methods of property damage, nuisances and such other things that can now be assessed in an economic fashion.

In noting the scope, we wanted to point out that, necessarily, engineering research in air pollution considers economic aspects to what might be called the "Nth degree." We feel that many of the problems in air pollution are the result of well-intentioned plant engineers who failed to consider the engineering aspects of pollution. Many well-designed processes, from a product or material standpoint, turn out to be rather poorly designed from the community air contamination standpoint. We are well aware of the fact that many of the problems which we now consider critical could be solved without further research by the application of well-known control principles.

However, in many cases it must be recognized that these are expensive, or are interpreted to be expensive by the operator, since he cannot see any immediate "return". In many cases, he never gets it.

So one point that we arrived at, which we thought was worth a separate heading, came from one of the university members of our group; and, that was the need in our area for basic creative thinking. We might put this on a basis that would correspond to a "Princeton University Institute of Advanced Study". We need to do some thinking about where we are going in this area of engineering control and what we need in the way of study projects. We want to point out the need for an engineering perspective to observe the processes. As we point out, this is one of our major engineering interests and a great need. So, while it may sound like a platitude, we think that this type of project, to which it is difficult to assign numbers, is the sort of thing that should be established to go along with basic thinking on this whole problem. We want to stimulate that type of project, and though we may not even have an individual or group capable of doing it at this time, we certainly ought to look for one.

We pointed out that there are two fundamental engineering approaches; first, the elimination of the contaminant at the source through modification of the process or materials handled. A lot of problems can be eliminated before the contaminants leave the operation. And, secondly, after they have been created, we feel strongly, as we always have felt in engineering control from an industrial hygiene standpoint, the importance of getting right at the source. We do not want to wait until contaminants appear in the atmosphere in large and diffuse quantities and then begin worrying about evaluation and control. Again, we had to make a division into categories of what is needed immediately and what is needed later. We had pressure from the West in terms of the Los Angeles situation, and pressure from the remainder of the group who were east of the Rockies. The latter group believed that we should consider the general problems of the East in items such as effluents from combustion operations, which are a major source of particulate contamination, acid gases such as sulfur dioxide and trioxide, and oxides of nitrogen and the hydrocarbon problem. These represent a perspective of the type of pollution that exists in almost every area. The other item we thought deserved immediate attention involved specific area problems. One can pinpoint these and state that almost every area has some of these items; for example, petroleum refining, chemical and metallurgical industries.

We considered that we should divide our problems into two groups, those which can be attacked in the field and those that can be handled in the laboratory. Naturally, we expected some relationship between these two. We also knew we could not operate independently, without overlap, because of our colleagues in the Aerosol and Instrumentation group; I would like to point out, however, that there was unfortunately no contact or cooperation with these groups in our case. We did not have the time to sit with the other group to find out what they were discussing; and, I found this morning, that some of the things that we propose, they are most anxious to do. So we are glad to give them the opportunity to do so.

The first thing we wanted to do in the field, as mentioned in our Report, was quantitative categorizing of effluents from combustion processes and determination of the formation rates of nitrogen oxide production from various processes, a rather broad study. We discussed some of the ramifications of this problem. For example, we wondered if we did not need a catalogue of some of these emissions. This has been proposed as one of the things that is needed. In overall thinking about the matter, we would propose that, where the developments allow, efforts should be made to make the catalogue complete.

In the laboratory work we emphasize the importance of test gas and aerosol production and preparation. And, in this area, of course, we overlap with the previous report. But we feel that in order to solve problems of collection, recovery, or the removal of the contaminants, we must know something about preparing the aerosols that are involved. So, again, I point out that if the other group is willing to do this, we would appreciate their efforts.

The studies of process modifications, I think, as do several members of the Committee, is a fundamental approach. Let us get back to finding out about the effect of conversions from dry grinding to wet grinding, from one type of material to another, which may influence the character and the amount of effluents and, in many cases, eliminate them. This is a rather large segment of a creative thinking project. Somebody should sit down and find out how we can pilot this type of experiment in the laboratory and review all the unit operations that may be involved with regard to contaminant production.

The last item under the laboratory group which we feel needs more immediate attention is the fundamental treatment of gaseous contaminants; and we have delineated these in terms of the type of problem and the general area; namely, the products of combustion and need for collecting these at an economic rate. We felt that there were certain requirements that we had to have before we could accomplish engineering research, and in this case we have indicated three major items. In the back of the report a number of less urgent items are listed that would be desirable to have studied.

The latter portion of the Report, which I will not go into in any detail, presents a compilation of all of the ideas that we generated in our deliberations -- areas in which we believe further work is needed. But, let me point out that we did not try to put a price on any of this. We feel that what we have listed here is the work that is not being done and we have placed them into two groups: 1) urgent priority, and 2) those which it would be desirable to have in the near future. In terms of effort, we feel that the engineering aspect is one of the major efforts because we may help to eliminate some of the other problems by source control -- and this we have emphasized.

I would like to thank all the members of the Committee who participated in submitting ideas and comments.

MR. STERN: Is there any comment or are there any questions that you would like to ask at this time? If not, is there anybody

associated with this group who objects to the publication of this report? (No objection). We will now go to the report of the final of the four non-medical working groups, that of the Meteorological Panel, and call on Dr. Wendell Hewson of the University of Michigan to report.

DR. E. WENDELL HEWSON: (The text of the Report appears in Appendix "A".) I would like first of all to express my personal appreciation for the efforts of the Meteorology Committee and the steering subcommittee which did so much of the work that you see presented in this report. I think, considering the size of our group, there was very broad participation in our discussions. Secondly, this report presents the results of a great deal of distillation of ideas in an attempt to arrive at the essence of the meteorological problems which are fundamental in air pollution studies. This has meant that, wherever there was an alternative of expanding or contracting a statement, we preferred to shorten it.

Some of these recommendations may seem to be rather imprecise. This again was deliberate. We avoided specific definitions of the terms used. This was not because we were unwilling to face the problem of definition, but with the time available it would have been unwise either to delineate these areas too much or to break them down into too many subdivisions.

You will find some overlapping of our proposals with those of the other Committees. These areas of overlapping are areas in which meteorologists are very much interested in obtaining information; this is our way of saying that we need such information so badly that meteorologists should try to obtain it if others do not take steps to do so. And, I would like to support the suggestion of Dr. Rogers for more joint sessions. He mentioned the period of about an hour that our two groups had together, which proved to be very valuable in promoting mutual understanding.

We did not assign priorities to these topics for research that we have listed, despite our efforts to do so. It soon became clear to us that there was insufficient time to draw up a meaningful list of priorities; so, the attempt was dropped. Nor were we able to specify the amount of money required for these various areas of research needing attention; the total sum would be, however, very substantial.

Our report consists specifically of two main sections. The first part presents a number of observations of a general nature which should be made at this time. The second portion lists the specific broad areas of research that we feel need attention.

As a matter of procedure, since the steering subcommittee and the whole Committee worked on this report, I will read it over without a break, and if we have any time left, perhaps I can elaborate or answer questions. (At this point Dr. Hewson read the report. (See Appendix "A".))

There are just one or two comments I might add. In the first item we are not referring to meteorology alone: "The design of a major air pollution investigation." We are thinking of two things. First, the desirability of all the disciplines involved in air pollution cooperating closely to design a comprehensive air pollution investigation for one or two representative cities. We feel it is so important to get together to design such an experiment that we should emphasize it by "spelling it out" as a separate item. The second point is the implementation of this design to obtain results that will be sufficiently general for application to other cities without large and expensive programs of measurements at each.

With regard to the item entitled "Investigation of self-purification processes in the atmosphere", there is one thing of broad general interest which has not come up. Evidence has been presented which suggests that we may be changing our climate by our industrial activity, and more specifically by the carbon dioxide released to the atmosphere as fossil fuels are burned. If there is any possibility that we are thus changing our climate, the matter is of enough national and international significance to warrant further investigation.

MR. MANOS: I would like to stress three of these recommendations which are especially important to the medical side of this program, as one of the users of the findings that would come out of such studies. I am referring to Items 11, 16 and 17, which concern the development of a pollution potential index, on the interpretation of climatological data, and the probability distribution of concentrations. We hope you have the answers right now, but we realize that in order to get these answers one has to go into all the other areas recommended here.

DR. HEIMANN: Would this be in your group for consideration?: The desirability of getting further information on artificial rain production as a method for hurriedly "washing out the air" in acute episodes of air pollution.

DR. HEWSON: The question of rainmaking was mentioned, but not in this regard. There are two difficulties to be faced. In the first place, it is not established that significant amounts of rainfall can be induced artificially, even under

favorable circumstances. Perhaps more serious is the fact that acute episodes, as in the Meuse Valley and at Donora, Poza Rica, and London, tend to occur with anticyclonic inversion conditions which inhibit cloud and hence precipitation development. At the present time the prospects of stimulating the atmosphere to wash out pollution in such acute episodes are not bright.

MR. STERN: Is there anybody who sees any reason why the report of the Meteorological Panel cannot be published? (No objection).

MR. STERN: Dr. Stokinger, from the Occupational Health Field Headquarters in Cincinnati, is going to present the report of the Medical Panel.

DR. HERBERT E. STOKINGER: There was a different type of presentation in our session than has been related regarding the engineering group. There was a group of formal presentations of short duration, interspersed with discussions of future work. I will try to report as faithfully as I can on each of these aspects.

If I misrepresent or misinterpret any speaker's intent, it is unintentional.

The prime objective of the medical program of the air pollution work is the safeguarding of human health, and comfort. If that is accepted as the prime objective, then one of the chief means of attaining this safeguard is the development of threshold limits, or, what is more familiarly known as maximum allowable concentrations, for air pollutants. To develop reasonable limits for the protection of the health and comfort of man, demands development of objective tests of pretoxic signs and symptoms of effects of air pollutants, the signs and symptoms of minimal physiologic alteration of body function, organ function, cellular function, or enzyme activity, while the alteration is still reversible. This in turn requires information gathered from a variety of disciplines. These were represented at the medical sessions in the form of pharmacology and toxicology, biochemistry, physiology, tissue culture, and cancer studies.

These are the objectives in the area of work that the medical program is operating under. Now, I will attempt to break down the significant contributions toward these goals, according to the disciplines under which they were presented.

It is, I believe, axiomatic that the medical program will be no better than the choice of substances that is made for study. The time, it appears, is now past for having a plethora of suspect compounds to study. The main reason for this is that the main groups of compounds of obvious importance are already under study, as you will hear about shortly.

If the program is to advance, it requires guidance from the air analysts to indicate the types of compounds for a particular study. In this connection we were fortunate in hearing from a number of speakers, the first of whom was Dr. Chambers, who related, among other things, the positive identification of the formyl radical. This is an introduction of a rather new concept; and might I just throw these on the board in addition to the others that are equally new.

He said the formyl radical advisedly because he felt that the formic acid is actually adsorbed onto particulate matter to create a greater response than is observed from the predictable or measured concentrations in the atmosphere, and it does not agree with kinetic reaction studies that this result would occur physiologically.

Another type of compound that he suggested might be worthy of medical attention was carbon suboxide, an unusual type of compound. It is highly irritating. According to his report, it appears in measurable amounts from the presumably incomplete burning of incinerated material and from combustion engines.

The third important compound which should require medical attention, it seems, is the newly substantiated peracylnitrite, which can be represented as a general group of substances where R represents the peracyl group. It has a highly oxidized carbon in it, to give oxidative effects. In addition, it has the nitrous oxide group for extra effectiveness as far as the physiological response is concerned. This compound has been the subject of attention of the group of workers at the Franklin Institute and is now, beyond question, identified to have a structure at least approximating this. (Points to blackboard).

All these substances are very potent respiratory tract irritants, and I think they deserve attention as soon as we can give it to them. In this connection, Dr. Fisher has selected a group of compounds along this line for Dr. Deichmann to study in the near future at Miami University.

Dr. Chambers also called attention to a new facet of the eye irritation problem, another type of irritant not related to solar action. In other words, we commonly think of the accumulation of the irritant type of smog in Los Angeles as occurring through the agency of sunlight; but, he called attention to the fact that there is also another type of irritant, presumably not oxidant in nature, that occurs in the dark phases of our existence, when the sky clouds up or right after dark. This directs attention to the search for another type of irritant that is apart from the peroxides, the ozonides, the peraldehydes and these other compounds that we are currently thinking about.

Dr. Chambers also spoke with some cogency on the need for giving important consideration to some other enhancing factor for the high activity of irritants and, as I referred to before, the extreme activity of the formic acid at such trivial concentrations cannot be accounted for on reaction kinetics. The suggestion is that this activity is enhanced by adsorption onto particulate matter, the idea being, of course, that if this particulate matter is a carrier for this gas, which otherwise would be absorbed in the upper respiratory tract and therefore cause less havoc, if it is adsorbed on a particle it will be carried deeper into the lungs where more serious injury will result. In evidence of this concept he noted that this increased irritation is associated with lowered light transmissivity of the atmosphere in general. The next point of evidence, he added, was that engine oil experimentally added increased irritation to some of these respiratory irritants.

Obviously, research is needed in this area in the medical field. In this connection Dr. Svirely announced he had some plans for immediate trial of some of these respiratory tract irritants along this line.

If the suggestion of Dr. Cadell, quoted by Dr. Chambers, that the organic free radicals are present in the air, is correct, then this is based on the fact that the half life of some of these free radicals is far longer than had been previously thought. Another possible reason is that under certain circumstances these free radicals have the characteristic of self-propagation and increment; that is, the destruction of one free radical results in a generation of two more. In this connection Dr. Saltzman showed, through a nice piece of theoretical reasoning, that such materials at the very low concentrations of smog, would, indeed, have half lives of appreciable duration; and then he went on to demonstrate the presence of several free radicals, of one, two,

or three carbon atom chain length from the reaction of ozone on hexane. These reactions were carried out at "air pollution concentrations" and were examined and identified by means of a mass spectrograph made especially for the study of this type of short-lived free radical. Here, then, is a host of possible new substances which should fascinate the imagination of the biologically trained. The added fact that free radicals are currently believed to be the prime factors in x-ray irradiation injury, and to play a role in the step-wise process of cancerigenesis, greatly enhances the importance of these free radicals, to my way of thinking, and accordingly their biologic testing should not be delayed.

In this connection also, Dr. Floyd demonstrated, with the use of hydroperoxides and peroxides, that there was a marked distinction in the toxicity of these closely related groups. The peroxides have the general structure of $R-O-O-R$. The hydroperoxides are very closely related but have hydrogen in place of one of the carbons. It is this type of substance that, in the preliminary screening toxicity study on a small group of these peroxides, showed that the hydroperoxide is by far the most potent of the two. These were also identified by the mass spectrograph in Mr. Saltzman's study. Now, this is an important point; but I think we need to determine whether or not the hydroperoxide is more important from a chronic toxicity angle. I think it should be made clear that acute toxicity effects may not necessarily bear any relationship to the chronic toxicity. They may involve entirely different mechanisms due to accumulation or inability of the body to handle repeated insults.

Another informative contribution to the subject of air constituents was made by Dr. Tebbens, who brought a great deal of information on the sources of the all-important air-borne carcinogens. To date he has identified 90 different structures of polycyclic hydrocarbons, now termed arenes; and, these appear universally from the combustion of gaseous fuels such as methane, propane, butane, butene and acetylene, and probably others that he has not had a chance to test; but the combustion, of course, must be incomplete. If the combustion is complete, obviously there is complete burning to CO_2 and water. He has confirmed the presence of the potential carcinogens from four other sources; from solid fuels, from auto exhausts, from the air of Berkeley, California, and five liquid fuels, although in this latter group they were produced to a lesser extent. Dr. Tebbens felt, if I sensed his report correctly, that there are present in the combustion products innumerable other substances of lower molecular weight whose activity we know nothing about, and these need to be investigated.

Another important contribution, I think, that Dr. Tebbens made was that he identified the oxidation products of the polycyclic compounds as well as the polycyclic hydrocarbons themselves. The importance of this is the growing evidence that it is not the hydrocarbon itself but its oxidation product that is the "starter" in the carcinogenic process. You can see that if the structures that he has identified are shown to be identical with those isolated from the body oxidation, he has demonstrated a carcinogenic step one point nearer the goal of its ultimate activity, which is cancer production.

In this connection Dr. Kotin detailed in a beautiful way the physical and chemical factors that determine the development of the cancerous process. Whether it is an aromatic polycyclic hydrocarbon or a lower molecular weight oxidized aliphatic irritant, elution of the carcinogen is necessary in the process of the development of the cancer. The reason for this statement is that he found, through a series of experiments of great nicety, that bonded carcinogen, that is carcinogens bonded to soot with which it is commonly associated and from which it is derived, is absolutely inactive in the host. It is not until after this material has been eluted, through normal processes requiring some fat solvent material that will dissolve away this carcinogen, that the cancer process starts.

Next, (he quoted evidence for this) the particle size of one micron is the most effective for traversing the air passages and the nose. Now, this requirement of elution, separation from the inert carrier, is especially appealing in another connection, namely, in explanation of the mode of action of a cancer "catalyst". This is a topic that was not discussed but is of great importance because these cancer "catalysts" comprise a group of what any chemist or biologist would call absolutely inert materials. They are aliphatic hydrocarbons of six to twelve to sixteen carbon atoms in lengths. They are non-carcinogenic in themselves; but, when applied subsequent to one application of the carcinogen, can result in a more rapid production of cancer. These materials are fat solvents and will dissolve these carcinogens and aid their transfer "into" the cells. They also have the other interesting property which will assist toward the production of cancer, and that is the irritative property. That is one of their characteristics. So I think he has hit upon a point that may be of great importance to cancerigenesis.

Some of the ozonides, peroxides and epoxides are such fatty elutants and act at the same time to irritate and destroy sensitive respiratory tract linings. This, Dr. Kotin showed

with oxidized gasoline. The rate of movement of the cancerigen particle in the respiratory tract can be enhanced or stopped by paralysis of the cilia of the respiratory tract. Then mucous secretion increases. Later the cells are functionless; then desquamation occurs. Cancer starts here in basal mambrane cells whose normal morphological defense is gone. This does not occur until the particles reach farther down in the bronchioles. Then there occurs an area of metaplasia and hyperplasia. Where impingement occurs, there is generally bronchial bifurcation. Recovered soot from the site demonstrates the absence of the carcinogen. Isotopic tracers indicate diffusion into the cells. The resulting cancers are true cancer, multiple, metastasizing and transplantable.

Dr. Gast reported some work with tissue enzymes, and selected for one of his major subjects of interest the polymorphonuclear leukocytes, the white cells. In the irritative processes, these are precisely the cells that migrate into the field of irritation and become a dominant factor at that site. He studied the leukocytes with such air pollutants as NO_2 -hydrocarbon, simulated smog; and he found that a 15-minute exposure of these leukocytes decreased their viability by 25 percent, at levels just slightly above air pollution concentrations, four to eight parts per million. He also got a concomitant decreasing oxygen uptake showing lessened viability of the cell.

Perhaps even more significant was Dr. Gast's demonstration of decreasing activity of smog for rat intestinal loops in atmospheres of high humidity. He compared the smog effects at high and low humidities and there was a decrease in activity here at the higher humidities. This finding has support from other work on the overall decrease in toxicity of smogs from plastic pyrolysis products, reported by workers in an entirely different field. Dr. Chambers also related a similarly decreased aerosol formation in high humidities as revealed by light scattering methods.

Dr. Pace, working with tissue cultures, explored this method as a means of demonstrating smog effects. He worked with HeLa cells, liver and fibroblasts, and showed considerable difference in susceptibility of these cells to the common pollutants SO_2 and NO_2 . He used, however, fairly high concentrations but found, interestingly enough, that at the lower concentrations that he tried (five hundredths percent, which is about 500 parts per million), there was a stimulation of cell growth. This is precisely what Dr. Kotin found when he exposed his trachea to some of these oxidants, and I think it is an interesting correlation and shows the possible contribution of this technique as a method of revealing biological effect.

A considerable group of studies on the physiologic effects of ozone were reported by members of the toxicology section of the Occupational Health Program. Ozone in this program has been given extensive investigation as a typical respiratory tract irritant important in oxidizing smogs. Emphasis has been placed on determining the mechanism of irritation and the mechanism of tolerance that is developed to this substance. The physiologic studies of Dr. Scheel have shown severely impaired respiratory function with progressively lowered respiratory volume and oxygen uptake on repeated exposures in the face of a demonstrated tolerance to many lethal doses of ozone. The effects of ozone on the cardiorespiratory system are being studied also by Dr. Matzen in open-chest experiments on dogs, in an effort to elucidate the manner by which ozone produces pulmonary edema. This is the fundamental mechanism of these irritants in the lung.

Biochemical studies reported by Mr. Mountain in ozone-poisoned animals have shown decreased activity of alkaline phosphatase and 5-nucleotidase in the lungs, and these are important enzymes. They are the enzymes that do all the work in the sugar turnover. The nucleotidases are the ones that build up the nucleic acid, which are important constituents of the cells.

Some factors were shown by Dr. Stokinger to modify the toxicity of ozone. These were: 1) that younger animals were more susceptible, 2) intermittent exposure, 3) premedication and pre-exposure lowered the toxic response of ozone, 4) exercise and respiratory tract infection favored the response. This is highly important in regard to workers in the highly irritant atmospheres, who should be permitted to sit down and rest periodically.

The most important finding we believe, however, in this ozone work that we have done, was the demonstration by Dr. Dobrogorski, our pathologist, that irreversible injury to the lung can occur from repeated insults over some period of time to ozone at concentrations that approximate air pollution levels. That is, repeated exposures to one part per million produced irreversible changes in the lung. I think it is the first demonstration where a common air pollutant at a level that is fairly reasonably attainable, can produce irreversible damage. This consisted of fibrosis of the terminal bronchioles and stenosis of the ducts, not admitting as much air, and emphysema which is a destruction of the alveolar walls and makes the bellows-like action of the lungs decrease; the elasticity disappears. You can get the air in but you cannot get the air

out. I have no doubt that other air pollutants of the irritative nature of which ozone is a prototype, will show similar changes. This is nothing peculiar to ozone alone.

Dr. Svirbely, in the first study to my knowledge of a mixture of air pollutants of which ozone was one, found that the addition of slight amounts -- amounts over one and a half parts per million of hydrogen peroxide -- can produce lethal effects in animals, whereas the same concentrations used singly in the same animals produced no injurious effects. Dr. Svirbely is starting on a program that is going to include ozone as a reactor with a number of other interesting possibilities such as thiols, the aldehydes and things of that sort, to see if the synergistic effect exists among the different pulmonary irritants.

Dr. Sim reported that in experiments in which he used sulfur dioxide on human volunteers, it was not an uncommon capacity of individuals to become sensitized to SO_2 if the exposure was sufficiently repeated. He also emphasized the marked enhancement in response to sulfur trioxide. Now, this time it was in high humidity atmospheres. The reason is, you get a droplet formation, particulates, and this involves a different mechanism. Humidity reduces toxicity of other types of smogs that are not soluble in water as SO_3 is.

Dr. Phair reported on three surveys of a group of selected cardiorespiratory cripples who were confined to their homes in Cincinnati, and data has been accumulated and readied for programming in a multiple regression analysis by an electronic computer, on four factors of the home environment; temperature, humidity, particulates, and SO_2 , along with some timed vital capacity tests, inspirational and expirational pressures, and newly-devised techniques of measuring carbon monoxide uptake. Although it is too early to say, it appears to him as though the carbon monoxide uptake showed decreases during the smog periods in Cincinnati in the last study, in contrast to what appears to be negative findings in the first two surveys.

There was a presentation or suggestion, by Dr. Lodge, on a simple classification of urban smogs on an oxidation-reduction basis where ozone, representative of Los Angeles, was one extreme of an oxidizing atmosphere; and London, with its SO_2 , was typical of the other extreme, the reducing atmosphere, with other cities presumably forming a spectrum in between. Now, this took a little "rough riding" in the presence of the medical

group, and the reason for this seemed to be chiefly because, as far as it was useful to them in this classification, these irritants acted irrespective of whether they had high oxidation potential or low oxidation potential. On the other hand, Dr. Lodge fought rightfully for his contention and mentioned that he has a problem of explaining to novices what types of smog exist. I think this may serve some useful purpose -- this simple classification which, of course, can be made more complex with time.

Now, as to future work, we did a little discussing of that through the Chairmanship of Dr. Fisher, and two questions for future programming received discussion at length. One was the study of eye irritation, what should be done about it and whether or not it should be done, and the second was toxicological screening of network samples.

Now, on the eye irritation problem it seems to me it was rightly stated by Dr. Chambers that, irrespective of its health significance, the idea was that anything that affects the public requires attention by groups such as ours. Questioning by Dr. Fisher produced a number of biological indices that I thought were highly interesting, referable to this human complaint. Dr. McEwen, who with Dr. Hine, has just received a research grant for investigation of eye irritation, suggested an oscillographic recording of nerve end firings as effected by smog. Examination of acuity under dim light conditions has been, I believe, in use by Mr. Krackow's group or related groups at the Army Chemical Center as another possible means of detecting this response.

Another suggestion was the correlation of visual performance to job performance. This was suggested by Dr. Schoettlin, and another was by Dr. Kotin. Kotin suggested that if tracheal ciliary response could be correlated with eye irritation, this might prove a highly quantitative measurement because it is possible to record this "down to the third decimal place". Now, these are all different approaches and I think there are some very interesting possibilities. The upshot of this whole discussion was that we were going to search for some physiologist who might be sympathetic to some of these ideas, and who, in turn is associated with an ophthalmologist.

The practicability of screening available collected air pollution samples from the National Network was discussed in great detail. There was a feeling that the work should be done and that there should also be some appraisal of what type

of results might be expected if it were done. There was considerable doubt, as you might gather, among some individuals as to whether the work would be worthy of the attempt and whether the approach might not be better spent in chemical analysis and identification, and, then present these chemical structures to the toxicologists to find whether they have some potential or whether they are merely materials whose toxic properties are described in any toxicologic textbook.

The result of the discussion of this on the positive side was this: That there seemed to be a real possibility of finding new or unusual responses from these air pollution samples by tests for carcinogenesis of these fractions that are appropriate to that work, or co-carcinogenesis in the neutral or aliphatic fractions. A number of cancer researchers expressed a great deal of interest in this field, and I think this would be the most outstanding use of these fractionated samples.

DR. FISHER: It is obvious from that presentation and results of the last couple of days that the major emphasis in this meeting was the toxicological studies of air pollutants. There are two other major segments of the program which are fairly well developed. Those have been discussed at meetings on pulmonary function and epidemiology. There will be more such meetings scheduled in the future, as will a conference of allergists.

MR. STERN: We have a few minutes that we can devote to a discussion of Dr. Stokinger's report or for questions you may want to direct to him.

I gather then that you accept Dr. Stokinger's report without any further comment, and I would just like to make a few closing remarks with regard to the sessions as a whole.

We first want to thank the Chairmen and the reporters for the five panels for the extra time and effort they gave in preparing and presenting the reports. Next, we thank those who were one step removed, the small groups that acted as steering committees and working groups to draft the reports we heard this morning. Lastly, we thank all of you for your contributions to the substance if not the particular wording of the reports presented.

In concluding this seminar, we solicit answers to the question of the value of a seminar such as this. This is the first time that, acting under what we believe to be the mandate of Congress to the Public Health Service to assemble and disseminate information and to foster and sponsor research, we have attempted to provide the physical facilities and the atmosphere in which a seminar of this type can be held. We solicit your opinion regarding this particular type of seminar.

There has been a suggestion in one of the panel reports that a Gordon-type conference might be highly desirable. I would like to give you a bit of history with regard to that. We had the feeling here about a year or so ago that a Gordon-type conference in the air pollution field would be valuable, and got in touch with the people who run the Gordon conferences.

For those of you who are unfamiliar with Gordon conferences, they are sponsored by the American Association for the Advancement of Science and are held in New England once a year with subjects selected, a group of people invited, and no publication of papers -- just an opportunity for people to get around a table and discuss advances in somewhat similar fashion to what we have done here. We are told that they have a limited number of days and limited space for holding such conferences, but that they were prepared this past year to split the water pollution conference into a water and air pollution conference. We felt that it was undesirable to dilute an existing water pollution conference by adding another subject, and suggested that the Gordon water pollution conference should be held intact, but that a separate Gordon air pollution conference be held as soon as it could be arranged.

If any of you feel very strongly that a Gordon air pollution conference is desirable, I would suggest that you let your feelings be known to the people who are responsible for the planning and conduct of Gordon conferences.

To get back to our own seminar we will want to find out from you whether this type of meeting should be repeated, what groups should be called together, whether we should adopt the policy of breaking the groups down as we have done here or, as some of the conference reporters have suggested, of bringing groups together for a greater cross fertilization of ideas. There has been a "speed-up" of all the activities here made obvious by the fact that everybody had to work so late. This suggests that if a seminar such as this is held again, more time may be needed. These are some of the things we have to know. We don't know whether this particular time of the year -- just preceding Christmas -- was a wise or an unwise selection. What is the time best suited to those of you who have university and research institute or governmental connections? Where should this sort of seminar be held? Is Cincinnati a good place? Do the facilities we have here lend themselves to the kind of session we are trying to hold? There has been a suggestion that some time should be spent by each of the panel groups doing some advance planning, before we get together. If this type of seminar is held again, should there be a steering committee meeting ahead of time to do some of the work before the main group shows up?

We attempted to give you some background material in air pollution research, the guide to research, and the index to the guide, so that you could have a point of departure. What was not done was to try to give you, in writing, a digest of what your answers should be. We thought it would be presumptuous on our part to tell you what your final reports should look like and what the outline of your findings should be. Sometime within the next few months you are going to get a communication from us asking detailed answers to these questions so that we can find out what you think about the need, frequency, and conduct of air pollution research planning seminars. We will digest your answers and let you know the consensus of opinion as to the future of this type of planning session.

DR. SILVERMAN: I would like to make one point here. The way I interpreted Dr. Hewson's remark, "a Gordon-type conference", was not to have the conference under the auspices of the Gordon conference group, and for that reason I would feel that if this type of seminar is desirable the complication involved would be to meet at a university campus at the off-time. This is exactly what they are doing in the Gordon conference. They just sit down in groups, or in mixed groups representing each area, and thrash out these problems without having to go through the formality of setting down all the items that were covered. In other words, the planning of the Gordon conference is on an informal basis. This status can be obtained in many other places, I believe, besides New Hampshire. The idea of a remote informal gathering, I think, is what was implied.

I would like to have had the opportunity to review some of the progress instead of trying to plan what should be done; because in our

planning we tried to avoid projects which were under way and looked for what is needed to fill the gaps. Sometimes you can make a miscalculation as to how wide the gap is when you don't have the benefit of the area covered. Some of these circles not only run tangent but they cross beyond the middle.

DR. GREENWOOD: I would like to suggest a combination, first to give the present status briefly and then provide some time for planning.

DR. LODGE: I should like to point out one thing which I think shows up specifically in the report of the Chemical, Aerosol and Instrumentation Panel, perhaps more than the others, which is this: It almost uniquely contains a list of extremely specific projects for which descriptions can be written, and it is therefore a rather "immediate" document. If you can actually describe a project, you are halfway to the answer, or further, in many cases. One danger that I hope we will avoid is any imputation that this list is complete, exhaustive, inclusive, or exclusive.

MR. STERN: Would anyone else like to be heard on the subject? Mr. Hanson, do you want to say anything in conclusion?

MR. HANSON: No. I think that you have said "thank you" to the whole group adequately, and that is all I would want to say.

MR. STERN: This makes the thanks official and ends the meeting.

AIR POLLUTION

research seminar

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Agricultural Committee

J. T. MIDDLETON, chairman

Air pollution significantly affects the agricultural economy of the country causing damage to animals and vegetation. Many plants respond to a concentration of air pollutants below that usually causing effects in animals, with notable exceptions such as carbon monoxide and hydrogen sulfide. Plants, therefore, may be useful indicators for the detection of air pollutants. The environment and genetic composition determine in large measure the influence of airborne toxicants to such an extent that, at present, it is difficult to prescribe allowable exposure to injurious materials.

The most important air pollutants affecting agriculture requiring additional research are the fluorine-containing compounds. The effect of compounds of bromine, chlorine, and iodine

are recognized, but they are not of immediate practical importance.

Perhaps next in importance are the oxidants, such as ozone, nitrogen oxides, and oxidized organic materials acting as a complex, which are believed responsible for smog damage to plants.

Ethylene and perhaps other low-weight, unsaturated hydrocarbons are believed to produce adverse effects on a variety of agricultural crops, including flowers and other ornamentals.

Miscellaneous organic materials as represented by aromatic complexes and phenolic compounds may have adverse effects on plants and require study.

Sulfur dioxide has long been recognized as an airborne contaminant responsible for specific injury to vegetation, and has been more extensively investigated than any other air pollutant.

Other airborne contaminants include the heavy metals such as mercury, lead, arsenic, and selenium as well as miscellaneous materials

such as hydrogen sulfide, mercaptans, ammonia, and carbon monoxide.

A description of some of the principal research needs follows.

Research on the effects of fluorides upon plants and animals has shown the gross symptoms resulting from the accumulation of fluorine-containing compounds in animals and plants.

A specific need exists for a study of the mechanism of toxicity and ultimate fate of diverse fluorine-containing compounds in both animals and plants. Both chronic and acute effects of fluoride accumulation are recognized. Particular attention should be paid to studies emphasizing the effect on agriculture of realistic concentrations of fluoride such as those found in industrial areas. Such studies should include the role of fluorides in skeletal development and organ function both for laboratory and farm animals. The effects of fluorides on cellular constituents, uptake, and translocation of fluorides, fate of fluorides in cells, and the nature of resistance and susceptibility in plant material are needed to elucidate the mechanism of action of this important pollutant.

An important area of research also needing attention is the effect of fluorides upon structure and physiological function in animals and plants. Such studies should elucidate the influence of fluorides on assimilation, digestion, and metabolism of the essential nutrients, as well as their effect on lactation, reproduction, and other vital processes in animals. Related studies in plants are required to determine the effect of fluorides on the assimilation and utilization of nutrients, photosynthetic activity, respiration, and enzyme systems, as well as growth and yield. It is also necessary that the degree of foliar necrosis, fluoride content of tissue, and rate of fluoride uptake be related to possible economic plant damage.

Environment and genetic composition materially affect the manifestation of fluoride accumulation in animals and plants. Factors which may affect fluoride toxicity in animals are different levels of nutrition, the presence of other toxicants and diseases, stage of development, pregnancy, and lactation. The response of plants to fluorides under different environments, including varied climatic condi-

tions, fertility, and water relations, requires investigation.

Some study should be made of the alleviation of fluoride effects in animals through management practices and therapeutic treatment.

Protective measures may be developed to reduce the effect of fluorides to sensitive plants.

Although methods for fluorine analysis in plant tissue are reasonably satisfactory at present, analytical methods for fluorides in air and animal tissue are somewhat less satisfactory and require further study.

Research on the influence of sulfur dioxide has been energetically pursued for the past 75 years. The results of these studies have resolved most of the problems affecting agriculture. Those that remain include the effect of sulfuric acid, aerosols, and sulfur dioxide on animals as well as the mechanism of their toxicity singly, together, and in combination with other contaminants.

Oxidants have been recognized as plant damaging agents for more than a decade in certain urban areas, notably in Los Angeles. Although some of the oxidants have been identified as ozone and nitrogen oxides, the agents responsible for most of the injuries to vegetation remain unknown though they are believed to be oxidized organics arising from photochemical reactions in the atmosphere.

There is a real need to develop analytical methods for the identification and determination of oxidants, and to determine the mode of action of the toxicants and their effect on metabolism and growth of plants and possible effects on animals. The gross manifestations of oxidants on plants are generally known.

Organic materials comprise a group of contaminants which have adverse effects upon plants and possibly animals. Ethylene and perhaps other low-weight, unsaturated hydrocarbons may be intimately associated with significant economic damage to orchids, floricultural and ornamental plants, as well as a variety of other agricultural crops. It is believed that ethylene is damaging to some plants at concentrations in parts per billion range, while no effect is recognized in animals at this concentration. A critical need exists for analytical techniques suitable for the detection and monitoring of ethylene and related materials.

Other organic compounds such as those found in the aromatic complexes have been implicated in certain instances of damage to vegetation but insufficient information is available to determine the exact nature of the material and its biological effect. It is presumed that a variety of miscellaneous organic materials might likewise be incorporated in this research area, such as growth regulators, fungicides, insecticides, and other agricultural chemicals.

Although mercury has a low vapor pressure, it has been known for some time to be an air pollutant, responsible for plant injury in enclosed spaces. The recent introduction of mercurial paints for use in greenhouses has renewed interest in this problem and emphasizes the need for a reevaluation of this toxicant. Similarly other elements including heavy metals are known to produce adverse biological effects; among these are arsenic, cadmium, lead, and selenium which in the past have been known to occur as air pollutants and should be reviewed periodically and kept in mind as air contaminants of possible importance to agriculture.

Other known air pollutants exemplified by hydrogen sulfide, mercaptans, ammonia, nitric acid, and carbon monoxide, while toxic to plants and animals, are not considered of importance to agriculture except under conditions of gross accidental discharge to the atmosphere.

Though they are recognized as important, this report does not include consideration of radioactive materials.

The air pollution research program in agriculture requires assistance and cooperation of chemical groups in developing needed analytical techniques, engineers for developing corrective and protective devices as well as air handling facilities for specific fumigation studies, meteorologists for assistance in understanding air contaminant dispersion patterns, and medical groups in developing and evaluating bioassay systems for identification and distribution of air pollutants.

The U. S. Department of Agriculture should augment funds available to existing research facilities and management services so that they may actively study from basic and applied points of view the factors affecting the occurrence and result of air pollution upon agriculture. The U. S. Department of Agriculture

with the Public Health Service should support liaison with private, industrial, and public research organizations.

The suggested research needs fall within the disciplines and competence of a large number of existing research organizations and they should be supported and encouraged to attack these problems.

Chemical, Aerosol, and Instrumentation Committee

LEWIS H. ROGERS, *chairman*

The projects listed below represent the combined efforts of some 30 scientists assembled to outline research areas and projects in the aerosol, chemical, and instrumental field which, in their opinion, require additional research. Taking cognizance of existing projects, the Chemical, Aerosol, and Instrumentation Committee attempted to develop projects not now being carried on. The order of presentation of projects is by subject matter and should not be taken to imply relative ranking according to importance. The group feels that sampling and analyses are such essential tools in all phases of air pollution that work in this field is needed before other problems can be attacked. Specifically, methods must be developed with precisely known accuracies, reproducibilities, and freedom from interference.

It is further recognized that an early start must be made in the study of certain basic problems because of the length of time required for their solution. During the discussions, it became apparent that one of the great needs of the field is for breakthroughs which fundamental research fosters.

In the following list short-, medium-, and long-range projects are designated by the letters S, M, and L, respectively.

Aerosols

Formation of Aerosols

Mechanism of smoke and fume formation including methods of modification (M).

Formation of aerosols during photolysis of polluted atmospheres (M).

Application of the particle-size spectrometer to nucleation studies (S).

Relationship of the composition of aerosols

to that of the feed material; for example, relationship of particulate sulfate to sulfur dioxide (L).

Changes accompanying the aging of particulate pollutants (L)

Development and evaluation of devices for the controlled production of aerosols, such as arc techniques and furnace methods (S).

Physical Properties of Aerosol Systems

Correlation of surface area with other properties (M).

Effect of aerosol shape on light scattering (M).

Dynamics of movement of particles in large volumes (M).

Development and evaluation of tracer techniques. Examples are:

- Magnetic materials (S)
- Natural germanium in coal, vanadium in oil (S)
- Micro-organisms (S)
- Automatic counting methods (S)

Sampling and Analysis

Behavior of particles approaching collecting surfaces (S).

Behavior of thermally conducting particles in a thermal gradient (S).

Development and evaluation of stack sampling equipment, such as high temperature aerosol cameras or counters, and particle size spectrometers (S).

Development and evaluation of new collectors, such as isokinetic samplers and wet collectors (S).

Application to aerosol analysis of special techniques. Examples are:

- Infrared reflectance (S)
- X-ray diffraction (S)
- Chromatography (S)
- Electrophoresis (S)

Interlaboratory comparison and statistical evaluation of analytical methods (M).

Gases

Gaseous Reactions

Photolysis of organic compounds in oxygen (M).

Rates and mechanism of elementary reactions in the photolysis of nitrogen dioxide with hydrocarbons in oxygen (M).

Photochemical reactions of effluents from known pollution sources, such as auto exhausts and incinerators (M).

Thermal reactions between ozone and unsaturated organic compounds (M).

Rates and mechanism of reactions occurring during incomplete combustion (L).

Nitrogen fixation in combustion equipment (M).

Sampling and Analysis

Collection of specific contaminants by means such as ion exchange resins and molecular sieves (S).

Development and evaluation of adsorption techniques for the concentration of air pollutants (M).

Development of techniques for the removal of specific substances such as water and carbon dioxide (S).

Development and evaluation of methods and equipment for low temperature condensation of pollutants (S).

Development of specific economical analytical methods suited to special needs. Examples are:

- Ozone (S)
- Acrolein (S)
- Halogenated carbonyl compounds in the p.p.b. range (S)
- Aldehydes (S)
- Oxides of nitrogen (S)
- Differentiation of sulfur compounds (S)

Development of bioassay methods such as enzymatic reactions and organism toxicity (S).

Interlaboratory comparison and statistical evaluation of analytical methods (M).

Development and evaluation of special non-specific analytical techniques. Examples are:

- Microbiological indicators for physiological effects (S)
- The vibrating reed surface potential apparatus (S)
- Special mass spectrometric techniques; for example, to determine parent masses and/or negative ions (S)
- Phosphorescence spectra and decay times for the identification of polycyclic hydrocarbons (S)
- Other physical techniques including NMR, EMR, and microwave spectrometry (S)

Development of special analytical techniques

for concentrations involved in work with odorants; for example, ultra long path infrared techniques (S).

Interaction of Gases and Aerosols

Photochemistry of organic and inorganic aerosols (M).

Heterogeneous reactions on surfaces (L).

The measurement of adsorption isotherms of pollutants (L).

Determination of the nature of the interaction of adsorbed species with the surface (M).

Meteorology Committee

E. WENDELL HEWSON, chairman

Our assessment of the present status of research on the meteorological aspects of air pollution indicates that progress on a wide front is being made. While very little overemphasis is noted, large areas of underemphasis are apparent.

Although the Meteorology Committee recognizes that it is not fully representative of all meteorologists working in the field, it nevertheless believes that the program of research which follows does outline in broad terms the general avenues along which further progress must be made. To pursue fully any of the particular subjects for research given here might require a number of research projects. We have deliberately kept the statement of each subject general in nature in order not to circumscribe unduly the necessary scope of possible effort. We have with equal deliberation avoided specific recommendations concerning advanced techniques of data transmission, data processing, and so forth, under the assumption that appropriate tools will be used in each case.

The committee recognizes that its recommendations undoubtedly will overlap some of those presented by other committees at this seminar; also, that, the solution of many air pollution problems will require the cooperative effort of several groups. Perhaps a Gordon-type conference would be desirable to further the coordination of effort among the several disciplines. (A Gordon-type conference is an informal research review conference held under the auspices of the American Association for the Advancement of Science.)

Apart from the particular research subjects listed, the committee discussed several activities which might enhance the growth of understanding of the meteorology of air pollution problems. Among these might be especially noted: (a) the widest possible dissemination by an appropriate agency of present meteorological knowledge of atmospheric dispersion; (b) compilation and review of knowledge of the various aspects of air pollution meteorology, augmented by a broad reevaluation of practical applications and incorporated into a monograph summarizing the field and indicating research requirements; (c) further development of a centralized national network to collect data on air quality which is directed toward better establishing the trend of air pollution; and (d) evaluation of available methods of meteorological measurements for their applicability to community air pollution studies. With respect to items (a) and (b) above, it is recognized that knowledge of recent developments in the field is not shared widely, even among practicing meteorologists. The committee welcomes the fact that some pertinent information previously classified may soon become generally available.

Recommended Research

General

Design of a major air pollution investigation.

On the basis of the above design, organization of a comprehensive and definitive urban area study.

Instrumentation

Development of simple yet adequate meteorological instrumentation and methods of analysis of resulting data.

Development of inexpensive tracer techniques for meteorological use.

Model Studies

Development of scale model techniques for simulating atmospheric diffusion processes as related to area sources.

Development of methods for simulating terrain influences on air flow patterns.

Physical Processes

Studies of nucleation, atmospheric transmission, and absorption of solar radiation.

Investigation of self-purification processes in the atmosphere.

Dispersion

Fundamental research on atmospheric turbulence and diffusion.

Study of persistent atmospheric conditions which tend to produce severe pollution.

Development of pollution potential indexes.

Investigation of the structure of the lower atmosphere in relation to basic diffusion processes, especially in urban areas.

Research on pollution from remote sources.

Theoretical studies of diffusion from large area and volume sources.

Study of the influence of heat sources and sinks on air pollution patterns.

Development of methods of interpreting climatological data for air pollution studies.

Investigation of the application of the methods of multiple-time series analysis, information theory and general stochastic procedures to the prediction of the probability distribution of the concentration of atmospheric pollution at a point.

Engineering Committee

LESLIE SILVERMAN, chairman

The objectives of engineering research in air pollution are to investigate, develop, and appraise mechanisms, devices, and processes for the control of air pollution and to define, measure, and evaluate air contamination from all sources. Control in this context encompasses the prevention of air contamination which may create a nuisance and which may be injurious to property, vegetation, animals, and human health and welfare.

Engineering research in the area of air pollution inherently includes economic considerations of control or elimination of contamination at the source. Many occurrences of air pollution result from a failure to consider those factors influencing air pollution. In this regard may be cited the fact that many otherwise well-designed processes consider only the product requirement and operational efficiency rather than the contaminants that may be discharged to the atmosphere as a result of the process. It is the opinion of the Engineering Committee

that this phase of the problem should be directed to the attention of those engineers who are responsible for process design.

The committee is aware of the fact that many of the problems now considered as critical can be solved without further research through the application of well-known and well-established control principles. However, it should be emphasized that one of the major phases of engineering research must be directed toward economic methods of control.

Considerable emphasis in air pollution control pertains to the effects of contaminants on property and vegetation susceptible to direct economic evaluation. The committee has considered these effects in addition to the health aspects of the problem.

One of the fundamental needs is perspective in consideration of the entire problem. This can be obtained only by giving consideration to future requirements while appraising present research in terms of its potential application to existing problems. The committee recognizes the value of such creative thinking in regard to engineering investigation in the air pollution area and feels that it should not be neglected in establishing any long-range research planning. Since the solution of many of the problems in air pollution requires various technical competencies in the engineering field as well as an engineering perspective toward the evaluation of source characteristics and control requirements, it is felt that the engineer, per se, is particularly adapted to the assessment of the basic problem.

The committee recognizes the essential need for definition of the air pollution problem in terms of relationships between pollutants and ill effects, whether in terms of human or animal response, vegetation injury, or material damage. It is further recognized that the rate of progress in solving air pollution problems is dependent to a considerable degree upon the rate at which these relationships can be established.

There are two fundamental engineering approaches to the problem of control of contamination in the atmosphere, and emphasis in research should be directed toward both:

1. The elimination of the contaminant at the

source through modification of the process or materials handled.

2. Control of contaminants at the source by suitable devices.

It is the opinion of the committee that the needed engineering research should be in two categories:

1. Problems for which early or prompt solution is desirable and practical. Included in this category are:

a) Control of pollutants associated with effluents from combustion operations, namely, particulates, certain acid gases such as oxides of sulfur and nitrogen, and hydrocarbons.

b) Specific area control problems associated with the effluents from the petroleum refining industry, the metallurgical industry, and the chemical industry.

2. Problems of a less urgent priority.

The general research program, as discussed by the committee, considered two divisions of these categories, namely, field studies and laboratory investigations.

It became apparent in discussing engineering research that instrumentation plays an important role, both in identification of a source and its magnitude and in the evaluation of control equipment. Some of the needed engineering research requires the prior development of suitable instrumentation.

Instruments urgently needed for research and control include:

a) An instantaneous quantitative gas spectrum analyzer.

b) Particle-size analyzer for the 0.01 to 0.3 micron range.

c) A rapid mass distribution analyzer for particulates in gas streams.

Also needed are the following:

a) Simple fluoride gas-aerosol discriminator.

b) Simple stack effluent weight analyzer.

c) Light scattering analyzer for solids in gas streams.

Problems Needing Prompt Solution

Field

Pollution of the atmosphere from solid fuel combustion processes, we believe, possesses such magnitude and characteristics that a qualitative

and quantitative assessment of these contaminants is necessary. This is best accomplished through a fundamental field investigation of:

1. The characteristics of the effluents resulting from solid fuel combustion processes.

2. The determination of factors affecting the formation of oxides of nitrogen.

The solid fuel process effluent characteristic evaluation should include the determination of physical characteristics of the solids and the assay of the chemical composition of the liquid and gaseous contaminant discharges.

Laboratory

Test Gas and Aerosol Preparation

Preparation of gas and aerosol simulants is an important aspect of the development and appraisal of engineering devices for control of air pollution. A fundamental investigation of means and methods of preparing gases to simulate atmospheres and processes is desirable and a similar approach to the preparation of aerosols of various physical properties, such as shape, size, specific gravity, and surface characteristics, is essential. Such aerosol preparation information is fundamental to the study of the behavior of aerosols both within quiescent atmospheres and in dynamic gas streams and in the study of the performance of various mechanisms for removal of aerosols.

Studies of Process Modifications

One of the important problems in air pollution control from a fundamental standpoint is to investigate the sources of air pollution as related to the industrial process. We believe that essential studies in modification of processes and materials handling, from a basic formation of contamination viewpoint, are of major importance. Modification or alteration of a process so as to eliminate the contamination obviously eliminates the need for further control equipment, and may contribute substantial economies. We believe that there are many unrealized opportunities for attack upon air pollution by this strategy.

Treatment of Gaseous Contaminants

Additional investigation is needed in the study of the absorption, adsorption, and chemical conversion of gases in such concentrations

that they are a nuisance or hazard. Such studies should include basic approaches to control of acid gases, including oxides of sulfur and nitrogen, and to the elimination of hydrocarbon vapor emissions. Emphasis should be placed on development of economically feasible methods and processes.

Tabulation of Needed Research

Field Investigations

Process Evaluation—Effluents

1. Compilation of characteristics and rates of effluent emissions.
2. Assessment of internal combustion engine emissions (urgent).
3. Determination of solid fuel process characteristics (urgent).
4. Appraisal of nitrogen oxide formation (urgent).
5. Relationship between transparent and opaque particulates.

Process Evaluation—Modifications

General investigation of process modifications for pollution control.

Control Equipment Evaluation

1. Establishment of incinerator design criteria.
2. Evaluation of filter devices.
3. Evaluation of inertial separators.
4. Determination of electrostatic precipitator performance.

Community Survey Techniques

1. Comparison of hourly dustfalls.
2. Acquisition of particle size data at ground level.

Laboratory Studies

Source Simulation

1. Preparation of test gases and aerosols (urgent).
2. Determination of properties, behavior, and methods of specification of aerosols (urgent).
3. Investigation of sampling methods (isokinetic).

Principles of Gas and Aerosol Control

1. Studies of process modifications (urgent).
2. Fundamental studies on filtration mechanisms.
3. Development of high temperature filter media.
4. Further studies on electrostatic phenomena.
5. Evaluation of droplet-type scrubbers.
6. Exploratory studies of less common mechanisms.
7. Fundamental treatment of gaseous contaminants (urgent):
 - a) Absorption and adsorption of SO_2 and SO_3 at low concentrations.
 - b) Oxides of nitrogen treatment and control.
 - c) Conversion studies including catalytic and noncatalytic combustion.